POLIOMYELITIS

Papers and Discussions Presented at the Fourth International Poliomyelitis Conference

Compiled and Edited for the
INTERNATIONAL POLIOMYELITIS CONGRESS



Philadelphia Montreal
J. B. LIPPINCOTT COMPANY

Editorial Note

The material in this volume comprises a record of the Fourth International Poliomyelitis Conference including a report of the opening session the banquet and the other functions The original presentations have been included in toto The discussions addresses and exhibits have been edited carefully and specially prepared for publication All the material delivered in several languages and transcribed by recording has been edited in the interest of accuracy and brevity

Library of Congress Catalog Card Number 49 9616

PRINTED IN THE UNITED STATES OF AMERICA

Dedication

Previous volumes of the proceedings of the International Poliomyelitis Conference have been dedicated to Franklin Delano Roosevelt whose leadership and inspiration gave birth to the Georgia Warm Springs Foundation the National Foundation for Infantile Paralysis and the International Poliomyelitis Congress This book is again dedicated to Franklin Delano Roosevelt

The Fourth International Poliomyelitis Conference was concerned primarily with the preventive vaccine now in use throughout the world which seems likely to terminate such epidemics of infantile paralysis as have threatened mankind in the past

This volume is dedicated also to Professor Edmond Grasset distinguished scientist in the field of public health and preventive medicine who for three years served as chairman of the committee which had charge of arrangements for the Fourth International Poliomyelitis Conference He died in September 1957 His untiring efforts his kindliness his leadership his knowledge and his special ability in securing co-operation from the representatives of many different nations will never be forgotten by those who had the privilege of working with him

Basil O Connor

Preface

The First International Poliomyelitis Conference was held in 1948 in New York City the Second in Copenhagen in 1951 the Third took place in Rome in 1954 So great has been the interest in the published proceedings of these conferences that the proceedings of the Fourth Conference held in Geneva Switzerland 1957 are now published in similar form

Copies of the first three volumes are available in practically all the medical libraries in the world Thousands of copies have been circulated to individual investigators and to institutions for medical research

The proceedings of the Fourth Conference include discussions on many different phases of poliomyelitis but the centers of interest were vaccination against the disease new information concerning enteric viruses that produce diseases simulating poliomyelitis and general considerations of viruses and of cultures of mammalian cells New information was made available concerning new technics in the diagnosis of infantile paralysis Other sessions were devoted to group and home care of patients with respiratory or extensive paralysis and the care of patients severely stricken by the disease The scientific exhibit was an important feature of the sessions and other aspects including the social functions are also covered by these proceedings

The editor and the publisher again extend their thanks to the contributors and to the educational institutions which collaborated in making possible the publication of the proceedings

Morris Fishbein M D

[illegible]
[illegible]

OFFICERS

President BASIL O CONNOR
Vice Presidents MORRIS FISHBEIN M D
RUSTIN McINTOSH M D
THOMAS M RIVERS M D
Secretary WM FRANK SNYDER
Treasurer HENRY P REINEKE
Executive Secretary STANLEY E HENWOOD

MEMBERS

Dr RENATO BOMFIM Brazil
Mr RICHARD A BRENNAN U S A
Mr FRANCIS BROWN U S A
Mr JOHN S BURKE U S A
Prof GIUSEPPE CARONIA Italy
Dr SVEND CLEMMESEN Denmark
Mr VINCENT CULLEN U S A
Prof ROBERT DEBRE France
Dr MORRIS FISHBEIN U S A
Prof E GRASSET
Mr ARTHUR KIRSTEIN U S A
Dr CARL KLING Sweden
Dr DAVID P C LLOYD U S A
Dr RUSTIN McINTOSH U S A
Mr CLARENCE G MICHALIS U S A
Mr JEREMIAH MILBANK U S A
Dr FRANK P OBER U S A
Mr BASIL O CONNOR U S A
Dr CARLOS E OTTOLENGHI Argentina
Mr L RAGOCZY Denmark
Dr THOMAS M RIVERS U S A
Dr HERNAN ROMERO Chile
Mr WM FRANK SNYDER U S A
Dr NORMAN H TOPPING U S A
Mr JUAN T TRIPPE U S A

The Fourth International Poliomyelitis Conference

at the Salle du Conseil General and the Aula of the University of Geneva

Organized by
The International Poliomyelitis Congress
with the co-operation of
The Institute of Hygiene and The University of Geneva

SPONSORS

The National Foundation for Infantile Paralysis (United States)
The Department for Labor Health and Public Welfare Geneva (Switzerland)
The University of Geneva (Switzerland)
The Federal Office of Public Health in Bern (Switzerland)
The Swiss Association against Poliomyelitis

HOSTS

The University of Basle
The University of Bern
The University of Fribourg
The University of Geneva
The University of Neuchatel
The University of Lausanne
The Swiss School of Economics and Administration of St Gall
The University of Zurich
The Swiss Federal Institute of Technology of Zurich
The Swiss Society of Internal Medicine
The Swiss Society of Neurology
The Swiss Society of Orthopedics
The Swiss Society of Pediatrics
The Swiss Society of Surgery
The Swiss Society of Microbiology
The Swiss Society of Physical Medicine and Rheumatics
The Swiss Society of Balneology
The Swiss Federation of Physicians
The Association of Physicians of the Canton of Geneva
The Society of Medicine of Geneva
The Touring Club of Switzerland
The Association of the Interets de Geneve

ENDORSING ORGANIZATIONS

American Academy of Orthopedic Surgeons
American Academy of Pediatrics
Dr Horace L Hodes
Dr James L Wilson
American Association for the Advancement of Science
Dr Jonas E Salk
Dr Wendell M Stanley
American College of Surgeons
Dr Frank R Ober
American Congress of Physical Medicine and Rehabilitation
Dr Herman J Bearzy
Dr Robert L Bennett
Dr Sedgwick Mead
Dr Jessie Wright
American Medical Association
American Physical Therapy Association
Miss Lucille Cochran
Miss Laura K Smith
American Public Health Association
Dr John P Fox
National Society for Crippled Children and Adults
National Research Council Division of Medical Sciences
The American College of Physicians
Dr Howard A Rusk
The American Neurological Association
Dr Howard A Howe
Dr Maximilian Silberman
The Association of American Physicians
Dr Thomas Francis Jr
Dr John R Paul
United States Army Office of the Surgeon General
Colonel Robert C. Blount M D
Colonel Adam J Rapalski M D

United States Navy Bureau of Medicine and Surgery
United States Public Health Service
Dr Alexander D Langmuir
Dr James A Shannon
Dr Joseph E Smadel
World Confederation for Physical Therapy
Miss M Margereson

CO OPERATING ORGANIZATIONS

American Public Health Association
J P Fox
Association des Paralyses de France
G Dingemans
Association of the Interests of Geneva
E Sandoz
Belgian League Against Poliomyelitis
L Schwartz
Canadian Foundation for Infantile Paralysis
C Gingras
D A Steele
Danish National Association for Infantile Paralysis
F Ammitzbøll
E Asmussen
A Frested
N I Nissen
J Ørskov
B Petersen
L Ragoczy
W Ritscher
Deutsche Vereinigung zur Bekampfung der Kinderlahmung E V
European Poliomyelitis Association
G Fanconi
Excerpta Medica Foundation
J Gauverein
M Fishbein
J E Minkenhof
Hadassah Israel Foundation for Infantile Paralysis
E Adler
International Labour Office
A A Bennett
Invalid Foundation
R K Jarvinen
M Martenson
Invalidity Fund of Workers
L Gunzburg
Israel Medical Association
S Rosenbaum
Israel Pediatric Society
R Kochmann
Swiss Association Against Poliomyelitis
P Vollenweider
League of Red Cross Societies
Z S Hantchef
Medical Research Council England
F T Perkins
F K Sanders
J O H Tobin
I N Sutherland
National Institutes of Health U S A
R Murray
J Warren
O K S Oslo
C M Aarsvold
Postgraduate Medicine
S Covet
Rancho Los Amigos Hospital
J E Affeldt
S A Poliomyelitis Research Foundation Johannesburg
E H Cluver
Society for General Microbiology London
C H Andrewes
State Serum Institute Helsinki
K Penttinen
I Petersen
United States Public Health Service
P S Brachman
A D Langmuir
E H Lennette
Vanderbilt Respiratory Center Nashville
R Batson
Western Regional Hospital Board Glasgow
T Anderson

CO OPERATING UNIVERSITIES

Aarhus University
C Nørby
Academie serbe des Sciences Belgrade
K Todorovitch
American University of Beirut
H Zellweger
College of Medical Evangelists
C W Dail
Academie R P R Bucarest
S S Nicolau
Hebrew University
H Bernkopf
Molteno Institute
K M Smith
New York University Bellevue Medical Center
H A Rusk

Paul Ehrlich Institut
O Bonin
Stanford University
C F Schwerdt
The Johns Hopkins University
E L Stebbins
Tulane University
J P Fox
Universitats Kinderklinik Leipzig
L Weingartner
Universitatskrankenhaus Ulleval Oslo
J C Ulstrup
University Ljubljana
M S Likar
University of Alexandria
A Shafik Abbassy
M Khalil
University of Bergen
D Scott
University of Buffalo
D T Karzon
B E Ol letz
University of Copenhagen
H C A Lassen
University of Florida
P H Vadheim
University of Genoa
C A Romanzi
University of Glasgow
T Anderson
J B Landsman
University of Gothenburg
C Jungner
E Lostrup
University of Illinois
C A Saxton
University of Leeds
J M P Clark
University of Louvain
J De Corter
University of Manchester
H B Maitland
University of Michigan
W W Ackermann
P A Locher
University of Oslo
P Holst
O Lahelle
University of Pennsylvania
W F M Limans
C W Rake
University of Pittsburgh
W McD Hammon
University of Southern California
O L Huddleston
University of Toronto
R C Parker

OFFICERS OF THE CONFERENCE

Honorary Presidents

Basil O Connor (U S A)
Prof A Gigon (Switzerland)

General Chairman

Prof Edmond Grasset (Switzerland)

Executive Secretary and Treasurer

Stanley E Henwood (U S A)

Committee of Honor

Mr Philipp Etter Federal Councillor Chief of the Department of the Interior
Mr Joseph Condrau President of the National Council
Mr Kurt Schoch President of the Council of States
Mr Alfred Borel President of the State Council of the Republic and Canton of Geneva
Mr Jean Treina President of the Department for Labor Health and Public Welfare of the Canton of Geneva
Mr François Bongard President of the General Council of the Republic and Canton of Geneva
Mr Maurice Thevenaz Mayor of the City of Geneva
Mr Jacques Iermoud President of the Municipal Council of the City of Geneva
Mr Arnold Sauter Director of the Federal Office of Public Health
Colonel Brigadier Hans Meuli Surgeon in Chief of the Swiss Army
Prof Alexander von Muralt President of the Swiss National Foundation for Scientific Research
Prof Andreas Werthemann President of the University of Basle
Prof Hans Robert Hahnloser President of the University of Bern
Prof R P Norbert Luyten President of the University of Fribourg

Prof Paul E Wenger President of the University of Geneva
Prof Edmond Grin President of the University of Lausanne
Prof Charly Guyot President of the University of Neuchatel
Prof Hans Fischer President of the University of Zurich
Prof Walter Adolf Johr President of the Swiss School of Economics and Public Administration Saint Gall
Prof Karl Schmid President of the Swiss Federal Institute of Technology Zurich
Mr Leopold Boissier President of the International Red Cross Committee
Mr Bonabes de Rouge Secretary General of the League of Red Cross Societies
Prof Ambrosius von Albertini President of the Swiss Red Cross
Prof Guido Fanconi President of the European Association Against Poliomyelitis
Prof Otto Gsell President of the Swiss Association Against Poliomyelitis
Mr Robert Briner President of Pro-Infirmis
Mr Leonard W Mayo President of the International Union for Child Welfare
Dr Etienne Berthet Director General of the International Children s Center
Prof Eric Martin Dean of the Faculty of Medicine of the University of Geneva
Prof Fernand Chodat Dean of the Faculty of Sciences of the University of Geneva
Prof Giuseppe Penso President of the International Association of the Societies of Microbiology
Mr Ahmed R Onat President of the World Medical Association

COMMITTEES OF THE CONFERENCE

SCIENTIFIC PROGRAM COMMITTEE

Dr Thomas B Turner *Chairman*
Dr John E Affeldt
Dr Robert L Bennett
Prof G Fanconi
Dr Morris Fishbein
Dr Thomas Francis Jr
Prof Edmond Grasset
Dr Oliver H Lowry
Dr Rustin McIntosh
Dr Frank R Ober
Dr A J Rhodes
Dr Thomas M Rivers
Dr Joseph E Smadel
Dr E L Tatum
Dr Norman H Topping
Dr Jessie Wright

Scientific Program Subcommittee

Dr John E Affeldt
Dr Robert L Bennett
Prof G Fanconi
Prof M R Francillon
Dr Thomas Francis Jr
Prof Otto Gsell
Prof Curt Hallauer
Dr Frank R Ober
Dr A J Rhodes
Dr Thomas M Rivers
Dr Joseph E Smadel
Dr E L Tatum
Dr Norman H Topping
Prof A Vannotti
Dr James L Wilson
Dr Jessie Wright

PROGRAM EXECUTIVE COMMITTEE

Prof Edmond Grasset *Chairman*
Prof F Bamatter
Dr Marcel Junod
Dr Andre Lenoir
Mr Arthur Lozeron
Prof Rene Mach
Prof Eric Martin
Prof Georges de Morsier
Mr Marcel Nicole
Prof Jean Posternak
Prof Jean Claude Rudler
Me Eric Sandoz
Prof Karl Walthard

SCIENTIFIC EXHIBITS COMMITTEE

Prof Jean Aime Baumann
Dr Robert L Bennett
Dr V Bonifas
Prof Pierre Duchosal
Prof Richard Extermann
Dr Morris Fishbein
Prof John Henry Oltramare
Prof Erwin Rutishauser
Dr M Sanz
Prof Hubert de Watteville

TECHNICAL EXHIBITS COMMITTEE

Prof Michel Demole
Mr Henry W Dunning
Dr W Geisendorf
Dr Albert Megevand
Dr Raymond Martin-du Pan
Prof Raymond Sarasin

ENTERTAINMENT COMMITTEE

Dr André Gautier
Mr Willy Greffier
Dr Pierre Koenig
Dr Thomas M Rivers
Dr François Thelin
Dr Thomas B Turner

RECEPTION COMMITTEE

Dr Jean Guinand Doniol
Dr Pierre Koenig
Prof J J Mozer
Dr Pierre Rentchnick
Dr C Edouard Schauenberg

Contents

GROUP AND HOME CARE OF PATIENTS WITH RESPIRATORY OR EXTENSIVE PARALYSIS

FINAL GENERAL SESSION

Reports of Official Delegates

Monday Morning July 8 1957

Chairman
DR ARNOLD SAUTER
Service federal de l hygiene publique
Berne

LEAGUE OF RED CROSS SOCIETIES
Dr Z S Hantchef

AUSTRIA
Prof Dr Karl Kundratitz

BELGIAN CONGO
Dr J P Delville

BELGIUM
Dr Pierre Recht

BRAZIL
Dr Oswaldo P Campos
Prof Jose Martinho da Rocha

CANADA
Dr F P Nagler

CHILE
Dr Hernan Romero

CZECHOSLOVAKIA
Dr Vilem Skovranek

DENMARK
Dr E Juel Henningsen

EGYPT
Prof M Diwany

FINLAND
Dr Mauri Larmala

FRANCE
Dr Lacombre

GERMANY
Bundesrepublik Deutschland
Dr Habernal
Deutschen Demokratischen Republik
Dr A Kukowka

GREAT BRITAIN
(England and Wales)
Dr W H Bradley

HUNGARY
Prof George Ivanovics

INDONESIA
Dr Soemiatno

IRAQ
Dr S D Taj-eldeen

IRELAND
Dr H O Flanagan

ISRAEL
Dr P Yekutiel

ITALY
Prof Giuseppe Penso

JAPAN
Dr Masami Kitaoka

KENYA
Dr J R Harries

LEBANON
Dr Joseph Hatem

NETHERLANDS
Dr H J Dykhuis

NORWAY
Dr Peter M Holst

PHILIPPINE ISLANDS
Dr Benjamin V Tamesis

POLAND
Prof Dr F Przesmycki

RUMANIA
Dr C Ionesco Mihaiesti

SWEDEN
Dr Gunnar Olin

SWITZERLAND
Prof Dr O Gsell

THAILAND
Dr C Purananda

UNION OF SOVIET SOCIALIST REPUBLICS
Dr Valentin D Soloviev

UNITED KINGDOM
(Colonies)
Dr W H Bradley

URUGUAY
Prof Enrique M Claveaux

YUGOSLAVIA
Prof K Todorovitch

LEAGUE OF RED CROSS SOCIETIES

AN OUTLINE OF RED CROSS ACTIVITIES IN THE FIGHT AGAINST POLIOMYELITIS

DR Z S HANTCHEF

THE RED CROSS AND THE FIGHT AGAINST POLIOMYELITIS

At the basis of all activities undertaken by ional Red Cross societies is the diffusion ong the population of humanitarian princi s and respectively the application of these nciples in the *prevention* and the *relief* of man suffering

For this reason the Board of Governors the hest authority and the General Assembly of League of Red Cross Societies considering it in recent years poliomyelitis has become a oblem of ever increasing importance in all rts of the world adopted the recommenda ns made by the League s Health Advisory mmittee and advised all national societies to rticipate as actively as possible in the fight ainst this disease

At the 23rd session held at Oslo in 1954 the ard of Governors of the League

recommends that National Societies should us include and stress in their health pro- ammes the precautions and measures which ould be taken generally and especially in times hen poliomyelitis is prevalent These are all ints which may easily with some appropriate planation be included not only in any Red ross health education programme for the lult public but also for the programmes of the nior Red Cross *As it is obvious that passive* imunisation such as for instance gamma obulin offering only temporary protection has ily a limited use active immunisation con itutes the final answer to the problem of pro- ction against poliomyelitis Therefore the oard of Governors is of the opinion that no ed Cross Society should undertake the production of gamma globulin as a method of preven tion of poliomyelitis only and that should any method of active immunisation be developed as may reasonably be expected Red Cross Societies should be encouraged to assist their govern ments as far as possible in carrying out their vaccination programmes

Considering that the need for qualified nursing personnel constitutes a major problem during acute outbreaks of poliomyelitis

and that equally important is the prob- lem of providing auxiliary personnel to give artificial respiration to the many patients suffer ing from respiratory paralysis during their trans portation to and their stay in hospital

the Board of Governors advises Red Cross Societies to train auxiliary personnel to cover this emergency and whereas in small communities sharp outbreaks are perhaps best dealt with by mobile teams organised on a regional level based on the principle of mutual assistance between sister Societies

recommends that as this task may exceed the powers of the Red Cross collaboration with other international health organisations working in this field such as the World Health Organi sation should be encouraged and

whereas besides the special medical problems within the rehabilitation in poliomye litis there are important social problems and these are from a Red Cross point of view the most important

considering that the importance of social assistance to chronic poliomyelitis patients can hardly be overestimated

advises therefore the National Societies to establish a close collaboration in this field with the public health authorities and the na tional associations against poliomyelitis in the respective countries

considering that only by supporting to the full extent of its powers the common fight against this social menace can the Red Cross be said to fulfil one of its fundamental tasks the prevention and alleviation of the suffering of humanity *

* Resolution No XIV of the Board of Governors 1954

Following up this resolution national societies have intensified their efforts in the fight against poliomyelitis and have been assisted by the League Secretariat and more particularly by the Medico Social Bureau. The latter has as its task the co-ordination of medico-social activities of the various national societies and the liaison with different international organizations such as the World Health Organization (WHO).

On two occasions—first the 23rd session of the Board of Governors in 1954 and second the Fourth International Poliomyelitis Conference in 1957—national societies have sent to the League reports on their activities in the fight against poliomyelitis. We have summarized these reports as follows:

OUTLINE OF ACTION TAKEN BY NATIONAL SOCIETIES

American Red Cross. All agencies involved in poliomyelitis *care recognize that the responsibility* for epidemic control is that of governmental *health agencies. A poliomyelitis planning* committee under the direction of the state *health officer functions in many of the states.* The American Red Cross and other agencies *concerned with the care of the poliomyelitis* patient are represented on this committee.

In poliomyelitis epidemics the total volunteer resources of the Red Cross are available to help *meet the needs in the community. Volunteers* transport nurses and family members to the *hospital, take convalescent patients to clinics* and maintain a system of reporting so that fami*lies can be notified daily of the condition of the* hospitalized patients. Junior Red Cross mem*bers furnish books, toys and other recreational* materials for children. An undetermined number of volunteer nurse's aides have been *assigned* to assist professional nurses in the care of polio*myelitis patients.*

Since 1943 the American Red Cross has been *co-operating closely with the National Foundation* for Infantile Paralysis and formal agree*ments have since been drawn up between the* two organizations. Thus it was that the American Red Cross agreed to act *as the recruiting* agency for the National Foundation for Infantile *Paralysis and has since 1949 co-operated with* the National League for Nursing in planning *in-service training programs for recruited nurses.*

Each local Red Cross committee maintains a list of nurses who agree to serve for a 3-month period if needed. Many of these nurses assist health departments in giving inoculations of gamma globulin or Salk vaccine on a volunteer basis.

Furthermore it is to be noted that the American Red Cross has co-operated with the National Foundation for Infantile Paralysis for the supply of gamma globulin from its Blood Transfusion Service.

Australian Red Cross. The State Department of Public Health has asked the central Red Cross Blood Transfusion Service *to undertake* the cleaning and the sterilizing of the equipment used *in the country's mass vaccination campaign.* It is estimated that 7 000 needles and *700 syringes will be used each day. The* State Department considers that there is *no other organization which could undertake this* work on such a vast scale. All costs for the *service will be borne by the Health Department.* As usual Red Cross workers will assist councils *and local bodies during the course of the campaign.*

Canadian Red Cross. *In the years preceding* the discovery of the Salk vaccine the Canadian *Red Cross carried through an extensive program* in the collection of blood and the manufacture *and the distribution of gamma globulin.* This field of activity was developed in agreement *with the Provincial Departments of Health* and incorporated many staff and volunteer workers.

At the Alberta Red Cross Crippled Children's Hospital 473 poliomyelitis cases have been *treated since January 1952.* The staff of the hospital is assisted by numerous regular and *part-time volunteers. In view of the marked* decrease in poliomyelitis and surgical tubercu*losis the building and the equipment have been* turned over to a local agency to be operated as *a general hospital for children.*

The Canadian Red Cross has also a water *therapy program* and 3 curative workshops for physiotherapy. Finally there is the Junior Red Cross Handicapped and Crippled Children's Fund through which needy children handi*capped in any way are helped with* the cost of treatment, hospitalization, orthopedic appliances, etc.

Czechoslovakian Red Cross. On the request of the public health authorities this society

will participate in a vaccination campaign for 2 000 000 children The Czechoslovak Government bought from Canada all the vaccine necessary for this large scale operation

Danish Red Cross This society i co-operating closely with another organization the Society and Home for Cripples According to an agreement between the two bodies polio patients are being received at the Danish Red Cross Convalescent and Rehabilitation Center at Hald near Viborg (Jutland) and are being treated by doctors employed by the above mentioned organization At the Hald Center there is a modern pool for the treatment of polio patients this was built with the support of the Danish Polio Association with which the Danish Red Cross is also in co-operation

Ecuadorian Red Cross Because of the small number of poliomyelitis ases in Ecuador this society has concentrated its efforts on the supplying of orthopedic appliances Moreover it is leading a movement promoting the giving of Salk vaccination to the population

Swedish Red Cross In 1949 the Royal Board of Public Health contacted the Swedish Red Cross in order to discuss the foundation of a society for action against poliomyelitis After further deliberations with doctors and Social Welfare authorities the National Association Against Poliomyelitis was formally presented to the publi in 1950 and given the tasks of (1) emergency preparations in view of anticipated poliomyelitis epidemics (2) convalescent treatment and rehabilitation of poliomyelitis victims (3) information and propaganda (4) s ientifi research (5) international co-op ration

From the proceeds of a fund raising campaign the Swedish Red Cross was able to increase the stocks of technical appliances for poliomyelitis patients in the so-called medical loan depots of which there are at present approximately 190 in the 24 Red Cross distri ts and to provide suitable material for diversional therapy The local Red Cross districts established special servi es for assistance to and rehabilitation of poliomyelitis patients such as home visits transport assistance various loans from Red Cross depots diversional therapy club activities swimming courses etc The National Association Against Poliomyelitis has undertaken to cover charges incurred by the Red Cross Rehabilitation Centers Members of Voluntary Aid Detachments and Red Cross auxiliary nurses have p rformed outstanding duties in connection with artificial respiration treatment and their work has been favorably acknowledged by the official medical authorities

The Swedish Red Cross is always prepared for emergency measures in case of an outbreak of poliomyelitis and Red Cross volunteers are being used as assistants to doctors and nurses in the present vaccination campaigns for preschool children and members of the armed forces

Swiss Red Cross This society is willing to participate as far as possible in the fight against poliomyelitis and consequently is an active member of the Swi s As ociation for Poliomyelitis Should an epidemic break out the Swiss Red Cross would make available its hospital services blood transfusion service (for supplies of gamma globulin) and voluntary personnel (auxiliaries nurses who have married and given up their profession) to assist the over worked nursing staff

Several local sections have established a service for the hronically ill The service has a qualified physiotherapist and can co-operate in the rehabilitation of patients

Thai Red Cross Up to the present Thailand has been left untouched by poliomyelitis epidemics However since 1954 the Thai Red Cross has admitted into its Chulalongkorn Hospital a total of 13 children suffering from infantile paralysis

French Red Cross Certain sections of this society have ambulances equipped with respiratory appliances for the transportation of poliomyelitis patients The French Red Cross also organizes reserve teams specialized in the care of poliomyelitis victims

Italian Red Cross This society has an institute at Malcesine on Lake Garda The center is equipped for kinesthetic treatment and the surgical treatment of inveterate sequelae of poliomyelitis

The institute also has the necessary material for diagnosis hydrotherapy (swimming pool baths etc.) massotherapy electrotherapy and the most modern installations for surgery It can accommodate about 150 infantile paralysis victims and more than 100 beds are available for infantile orthopedi cases

THE ROLE OF THE LEAGUE OF RED CROSS SOCIETIES IN TWO RECENT POLIOMYELITIS EPIDEMICS

Argentina In February 1957 an epidemic of poliomyelitis broke out in Argentina Some 2,300 cases were reported of which 10 per cent terminated fatally and on the average the victims were from 3 to 4 years old

A telegram was sent immediately from the League Secretariat offering help to the Argentine Red Cross

With the support of the Ministry for Foreign Affairs and the Association for Infantile Paralysis the Federal German Red Cross spontaneously sent a delegation of 3 doctors (2 pediatricians and 1 anesthetist) and 2 hospital nurses all of whom were Red Cross specialists in the care of poliomyelitis patients The delegation took with them an iron lung several respirators and certain medicaments

The Italian Red Cross also sent 2 iron lungs

The American Red Cross advised the National Foundation for Infantile Paralysis and contacted the United States Government An expert was then sent to Buenos Aires in order to survey the situation and 21 iron lungs were dispatched by air

The Czechoslovakian Red Cross sent a gift which was used for the purchase of a large motor conveyance for the National Institute for the Rehabilitation of the Handicapped in order to facilitate the transportation of patients to their treatment centers

A gift of hospital linen and bandages was sent to Buenos Aires by the Indian Red Cross

Meanwhile of course the Argentine Red Cross was taking a most active part in the fight against the disease From the start of the epidemic this society had sent nearly 200 nurses to various hospitals and rehabilitation centers Two iron lungs had been made available and the society made collections among the public for the purchase of 3 more It also collected clothes food medicaments books and toys

Hungary Following the recent outbreak of poliomyelitis in Hungary the Hungarian Red Cross has asked the League if it could arrange for the loan of 20 iron lungs and other respiratory apparatus as quickly as possible

In the early stages of the epidemic the delegate of the International Committee of the Red Cross at Budapest transmitted to the Hungarian Red Cross a quantity of gamma globulin

According to information given to the delegate of the International Red Cross at Budapest by the Hungarian Ministry of Public Health more than 750 cases of poliomyelitis have been registered A number of these are children 70 patients are seriously ill and a majority of these cases require respiratory apparatuses

The League has launched an appeal to a number of national societies believed to be in a position to loan such equipment to the Hungarian Red Cross Subsequently the British Red Cross Society has announced the dispatch of 2 iron lungs by air and the Federal German Red Cross is preparing to send 12 The Canadian Red Cross Society has offered to send if necessary gamma globulin supplies

In addition the Swedish Red Cross has decided to send 2 poliomyelitis experts Dr Jonas Lindahl and Dr Werneman to Budapest to survey the situation and determine needs On arrival in the Hungarian capital they will confer immediately with the Hungarian Red Cross the Ministry of Public Health and the delegates of the International Committee of the Red Cross and the League

AUSTRIA

Prof Dr Karl Kundratitz

Oesterreich ist in den letzten Jahren von keiner schweren Polio-Epidemie heimgesucht worden Die letzte grossere Epidemie war im Jahre 1947 mit 3 508 Erkrankungen und einer Mortalitat von 8 6 % Die Zahl der Erkrankungen sank dann z B im Jahre 1952 auf 198 um dann allmahlich wieder anzusteigen Voriges Jahr kam es wieder bis zu 1 018 Erkrankungen mit 92 Todesfallen

Seit 1954 ist es nun auch in Oesterreich moglich Epidemien virologisch zu untersuchen So konnte bei 4 Epidemien nur Typ 1 gefunden werden Bei sporadischen Fallen besonders bei meningealen Formen wurden die beiden anderen 2 Typen des Virus häufiger gefunden

Bemerkenswert waren voriges Jahr zwei Anstaltsendemien eine in einer Sauglingsstation mit 8 Erkrankungen bei diesen beiden Endemien konnte immer nur der Typ 1 nachgewiesen werden Bei einer Endemie dieses Jahr in einem Kinderheim konnte bei ungefahr 500 Klein Kindern die in einem direkten oder indirektem

Kontakt mit einigen Erkrankungsfallen standen ohne klinische Erscheinungen oder geringen Minimalsymptomen bei einigen Kindern in 80 / poliovirus wieder nur vom Typ 1 im Stuhle gefunden werden

Es stehen in Wien bisher nur 2 Untersuchungsanstalten und zwar das hygienische Institut der Universitat und die bundesstaatliche Untersuchungsanstalt zur Verfugung um Gewebekultur Untersuchungen durchzufuhren verbunden mit der Bestimmung neutralisierender sowie komplementbindender Antikorper

Von Bedeutung ist dass in Oesterreich jetzt oftmals andere Viruserkrankunge des Zentralnervensystems mit dem klinischen Bild einer Poliomyelitis ablaufen auch mit Lahmungen und Todesfallen Neben anderen schon b kannten Viren wurde im vergangenen Jahre auch ein neuer Mause pathogener Stamm isoliert

Grosses Interesse wenden die Gesundheitbehorden der aktiven Schutzimpfung zu Es konnte aus Mangel an Impfstoff noch keine allgemeine Impfung durchgefuhrt werden Es wurden aber bereits im Jahre 1955 mehrere 1000 Kinder mit dem Impfstoff der deutschen Behringwerke geimpft und in diesem Jahre ebenfalls bei 1000 Kinder mit dem Impfstoff der amerikanischen Fa Pitman Moore

Nun werden bereits die organisatorischen Vorbereitungen getroffen um freiwillige Impfungen in grosserem Ausmasse durchzufuhren da auch vom Nachbarland Ungarn eine Einschleppung zu befurchten ist

1954 hat in Oesterreich das Institut fur Haemoderivate die Produktion von Poliomyelitisvakzine begonnen (nach Salk) und diese durfte uns auch bald zur Verfugung stehen

Fur die verschiedenen Fragen die mit dem Gesamtproblem der Polio zusammenhangen haben die Gesundheitsbehorden in Obersten Sanitatsrat die massgebende beratende Korperschaft Fur Behandlungszentren mit den notwendigen Beatmungsapparaten ist vorgesorgt Fur den Transport stehen Rettungsautos vom Roten Kreuz mit Beatmungsapparaten zur Verfugung

BELGIAN CONGO

Dr J P Delville

Le fait saillant de la poliomyelite au Congo Belge est que cette maladie ne touche les congolais que dans le tout jeune age et ce avec relativement peu de degats alors que les populations blanches lui paient un bien plus lourd tribut et sont touchées a tout age

Depuis 1945 le nombre de cas de poliomyélite est en progression constante tant chez les congolais que chez les europeens avec cependant une incidence beaucoup plus elevee chez ces derniers

Pendant l annee 1954 annee d epidemie de poliomyélite pour laquelle nous disposons de donnees suffisamment precises l incidence de cette maladie fut de 4 pour 10 000 chez les européens contre seulement 0 64 pour 10 000 chez les congolais

Cette épidemie sevit plus particulierement à Elisabethville fin 1954 debut 1955 et nous avons pu l etudier de pres

Tous les cas sont survenus entre novembre 1954 et mars 1955 soit pendant la saison des pluies qui fut particulierement pluvieuse Il est a noter qu A Elisabethville les cas de poliomyelite surviennent toujours presque exclusivement pendant la saison des pluies

Pendant cette epidemie l incidence fut de 2 08 /o chez les europeens contre 0 43 /o chez les congolais avec une mortalite respectivement de 12 5 / et de 7,5 / des cas La mortalite fut donc egalement nettement superieure chez les europeens

La repartition des cas en fonction de l age reprise dans le tableau ci dessous merite également d etre signalée

	Congolais	Européens
De o à 2 ans	87%	25 0%
Mo ns de 10 ans	100%	6? 5%
Plus de 10 ans	0%	37 5%
Plus de 20 ans	—	33 0%

Pour une autre epidemie survenue à Bukavre en 1956 chez les congolais 88 / des cas avaient moins de 2 ans et 41 / moins de 1 an et aucun cas ne fut constate au delà de l age de 6 ans

Nous avons également pu faire l etude virologique de ces deux epidemies et il est interessant de noter que les 18 souches de virus poliomyelitique de l epidemie d Elisabethville et les 15 souches de l epidemie de Bukavre que nous avons pu isoler sur cellules Hela furent toutes du type 1

De ces deux epidemies nous pumes en outre

isoler 28 souches de virus Coxsackie dont 14 en association avec le virus poliomyelitique

Toutes les souches provenant de l epidemie d Elisabethville furent du groupe histologique A alors que l epidemie de Bukavre nous fournit des types A et B

Signalons que nous avons egalement peu apres isole du virus Coxsackie B a partir du liquide cephalo-rachidien chez deux cas de meningite aseptique

Alors que les cas de poliomyelites paralytiques ne nous ont permis que l isolement de souches de type 1 l etude des anticorps seriques montres que les autres types sont tout aussi repandus

Les resultats de l etude des anticorps seriques chez les congolais sont en parfaite concordance avec les constatations epidemiologiques

Les anticorps herites de la mere disparaissent pendant la 1r annee de la vie Mais dejà a partir de l age de 6 mois nous voyons apparaitre des anticorps d immunisation active et vers l age de 6 ans pratiquement 100 % des congolais possedent des anticorps vis a vis des 3 types de virus poliomyelitique

La vaccination antipoliomyelitique a ete entreprise debut 1957 dans les ecoles europeennes des grands centres mais sans que celle-ci soit rendue obligatoire

BELGIUM

Dr. Pierre Recht

SITUATION EPIDEMIOLOGIQUE DE LA POLIOMYELITE EN BELGIQUE DEPUIS 1954

Apres l annee 1954 au cours de laquelle il n y eut que 198 cas de poliomyelite la Belgique a connu en 1955 et 1956 deux epidemies successives qui ont fait en 1955 979 victimes et en 1956 1 038 victimes

Le total des cas de poliomyelite constates dans notre pays depuis 1940 soit pendant 17 ans est de 5 389 ce qui nous fait une moyenne de 317 cas par an pour une population de ±8 700 000 habitants soit 3 7 cas par 100 000 habitants

L epidemie de 1955 de 979 cas au cours de laquelle 34 deces ont ete constates represente une moyenne de 11 par 100 000 habitants L epidemie de 1956 de 1 038 cas avec 30 deces represente une moyenne de 12 cas par 100 000 habitants

Les variations saisonnieres de la maladie indiquent que les epidemies de 1945 et 1957 se sont surtout deroulees de juin a octobre celle de 1955 de juillet a decembre et celle de 1956 de juillet a novembre La maladie garde dans l ensemble un caractere de predominance estivo-automnale, le mois de fevrier connaissant le minimum de cas

L etude des groupes d ages touches par la poliomyelite indique que la periode de predilection se situe entre 0 et 10 ans

	Enfants de 0 a 10 ans	Adultes de plus de 20 ans
En 1952	68% du total des cas	13%
En 1955	80% du total des cas	11%
En 1956	70% du total des cas	15%

La poliomyelite reste surtout une infection infantile mais au cours de ces dernieres annees on a observe une augmentation du nombre des cas qui touchent les adultes la gravite des formes augmente avec l age et notamment les formes respiratoires sont de loin plus frequentes au dela de 20 ans

Quant a la distinction entre les formes paralytiques et non paralytiques les statistiques de notre pays indiquent qu en 1952 80 des cas signales etaient des formes paralytiques en 1955 78 % en 1956 63

Le pourcentage plus faible des formes paralytiques au cours de l annee 1956 est du vraisemblablement a deux raisons d une part une declaration plus fidele des cas de poliomyelite meme nonparalytique et d autre part l incidence au cours de l annee 1956 de nombreuses atteintes meningees dont certaines ont été sans certitude rattachees a la poliomyelite

En conclusion la poliomyelite dans notre pays est une maladie à predominance infantile dont le taux d endemicité se situe pour les deux dernieres annees entre celui de la diphterie et de la scarlatine et qui nous permet de classer notre pays parmi les nations favorisees Par rapport aux Etats Unis et aux Etats Scandinaves la frequence des cas est actuellement de 3 a 4 fois moindre meme en periode d epidemie

EPIDEMIE DE 1956 ET MENINGITE EPIDEMIQUE

De juin a decembre 1956 de nombreux pays de l Europe Occidentale ont ete touches par une epidemie de meningite aseptique

En Belgique l agent étiologique de 254 cas a ete identifie comme un virus ECHO apparte nant au type 9 *

Des echantillons de selles reçus de Suisse con tenaient aussi du virus ECHO du type 9 et la source isolee au Pays Bas a ete trouvee semblable à celle trouvee en Belgique

Ce virus etait aussi aisement decouvert et mis en evidence à partir du liquide cephalo rachidien qu a partir des selles

Les produits de gorge contiennent le virus mais uniquement quand il est recueilli au tout premier jour de la maladie

Certains virus ECHO belge type 9 se sont reveles etre pathogenes pour le souriceau quand ils sont inocules apres passage sur cultures de tissu L inoculation directe du virus à partir d echantillons de selles n a donne aucun sympto me Aucune de ces souches n a ete neutralisee par des serums de types connus

La meningite aseptique que nous avons connue en Belgique etait caracterisee par des symptomes meninges et etait parfois accompa gnee d exanthemes la pleocytose etait toujours marquee plus de 5 000 elements par mm La maladie possède un haut degré de contagio sité De nombreux exemples ont montré que tous les membres d un famille enfants et adultes peuvent etre atteints

ORGANISATION DE LA LUTTE CONTRA LA POLIOMYELITE

Nous avons organise en 1954 a l initiative du Ministere de la Sante Publique et de la Famille un reseau respiratoire belge destine a traiter les complications respiratoires de la poliomyelite Huit centres dont les 4 centres universitaires ont ete specialement equipes en appareils à respiration artificielle Un service d ambulances specialement affectees au transport des malades atteints de poliomyelite a ete organise avec un seul numero d appel téléphonique pour toute la Belgique En raison de l automaticite presque complete du réseau telephonique il est possible pour un medecin d appeler de n importe quelle commune de Belgique le service central qui se charge dès lors de prevenir le secteur de transport le plus proche du domicile du malade Ce service a fonctionne au cours des épidemies de 1955 et 1956 et a effectue plus de 500 transports

---

* ECHO t Type 9 (n w m mbe of Cox a k group
T p A?) a ause f r d m m n n, t Sc n
125 44 195

Chaque secteur d ambulance a un rayon de ±25 Km Les centres de traitement sont repartis de telle maniere que sauf pour le Sud du pays qui heureusement est peu peuple la duree maximum du trajet en ambulance est de 1 heure

Nous sommes persuades que cette initiative a permis de sauver de nombreuses vies humaines et que la mortalite de la poliomyelite et la gravité des atteintes en ont ete ameliorees

L Etat est intervenu tres largement dans l achat de l equipement des centres destines a recevoir des malades atteints de troubles respira toires Il est en effet difficile de faire supporter par un organisme d assistance local les frais d achat de poumons d acier ou d appareils respi ratoires à pression positive couteux alors que ces centres sont destines a recevoir des malades venant d autres regions Aussi est-ce le Gouverne ment qui a fait l acquisition des appareils et les a mis a la disposition des centres agrees

Des echanges de materiel et de personnel ont eu lieu en 1956 lors de l epidemie qui a provo que vers les mois d octobre et de novembre un nombre tres eleve de formes respiratoires

La formule que nous avons adoptee pour installer les centres de poliomyelite aigue est inspiree des recommandations parues apres le 2e symposium européen de la poliomyélite qui s est tenu a Paris en 1954

Nous pensons qu un centre destine a recevoir les malades aigus doit etre situé dans l enceinte d un hopital general et peut ainsi beneficier de l intervention des differentes specialites médicales intervenant dans la lutte contre les complications respiratoires

Il existe neanmoins en Belgique un centre prive qui est specialisé dans la poliomyélite et qui traite les formes aigues et chroniques

Mais 6 centres sont installes dans les services de contagieux et 1 dans un service de neuro logie situes dans des hopitaux generaux

L equipement respiratoire actuel de la polio myelite comprend une quarantaine de poumons d acier une douzaine d appareils Engstrom une vingtaine d autres appareils à pression positive et une dizaine de cuirasses

LA VACCINATION

Devant l importance du problème de l appli cation de la vaccination antipoliomyelitique le Ministre de la Sante Publique et de la Famille a constitue en avril 1955 une Commission mini

s adressent a des etudiants de l Universite du meme age 23 % seulement des sujets ont des anticorps contre les trois types

Un second exemple montre l influence des *conditions economiques et sociales* les enfants que ont fait l objet des examens prelimi naires aux vaccinations appartiennent a des milieux d ingenieurs de professeurs et surtout de medecins Il est remarquable de constater que ces enfants se revelent tres mal immunis s contre la poliomyelite et que meme a 14 ans 11 % seulement possedent des anticorps contre les trois types

La Figure 2 demande les explications sui vantes Troise courbes sont portees sur le meme graphique La premiere (traits pleins) a ete dessinee d apres les renseignements fournis par le Dr Recht au sujet des 1 078 cas de polio myelite survenus en 1956 elle donne selon l age le pourcentage cumule des cas de polio myelite sur la totalite des cas enregistres 45 % *avaient 4 ans ou moins 72 avaient 9 ans ou* moins 87 % avaient 19 ans ou moins

De la meme façon a ete etabli le pourcentage cumule des sujets exempts d anticorps (traits) et des sujets non completement immuni ses (traits)

On constate que les cas de poliomyelite se repartissent suivant la courbe des sujets exempts d anticorps plutot que suivant celle des sujets porteurs d une immunite nulle ou partielle Ceci indique que les risques de contracter une polio myelite cliniquement apparente sont surtout grands lors de la premiere rencontre ave le virus plutot que lors des deux suivantes et que par consequent la predisposition individuelle semble jouer plus que les facteurs occasionnels Lorsque le virus poliomyelitique fit son appa rition en Belgique lors de l epidemie de 1956 il se trouva en presence d une population ou les sujets exempts d anticorps se retrouvaient a raison de 43 % dans le groupe d age de 4 ans et moins or les ravages que la maladie causa furent precisement constates a raison de 45 % chez ces memes enfants de 4 ans et moins

Dans les groupes d ages plus eleves les *courbes divergent entre* 5 et 9 ans il y a 19 % de sujets sans anticorps mais 27 % de cas de poliomyelite Si cette divergence est significative elle est un indice que chez les sujets exempts

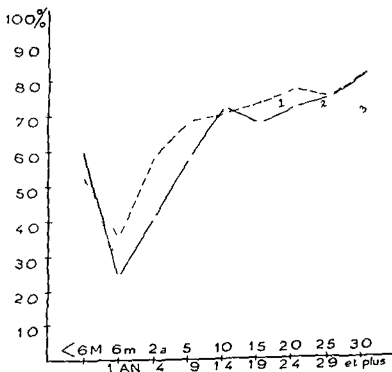


Fig 3 (*See text*)

d anticorps les risques de contracter une poliomyelite clinique augmentent avec l age Or cette divergence doit etre significative car ces courbes donnent des pourcentages cumules et convergent donc toutes normalement vers le point 100 /

Pour qu une divergence se dessine il faut que l un des facteurs se modifie avec l age ce facteur serait sans doute la receptivite individuelle aux complications nerveuses poliomyélitiques Le meme écartement entre les deux courbes se retrouve encore si ce sont les cas de poliomyelite de 1952 que l on porte sur les graphiques au lieu de ceux de 1956

Procedent à un autre calcul Olin en Suede[1] a demontré également que la meme rencontre avec un virus poliomyelitique comporte d autant plus de dangers que le sujet est plus age

Figure 3 montre la comparaison entre le pourcentage des individus immunises contre le type 1 au moins et celui des sujets immunises contre les types 2 et 3 au moins L immunite contre le type 1 est un peu plus precoce que pour les deux autres types En fait on devrait s attendre a une difference beaucoup plus forte car depuis trois ans que des recherches de virus polio sont effectuees en Belgique sur une assez grande echelle chez des malades paralytiques et chez des porteurs sains les souches de type 1 se retrouvent toujours dans 80 a 90 / de cas contre 5 a 10 quand il s agit du type 2 et du type 3

Or la meme discordance se retrouve dans beaucoup de pays il y a une predominance tres elevee de la souche 1 et une predominance peu marquee de l immunite contre le type 1 Ces deux faits ne peuvent être concilies qu en supposant qu'il circule parmi la population des virus de types 2 et 3 que nous decelons par les anticorps qu ils font apparaitre mais que nous ne pouvons mettre en evidence directement au laboratoire peut-etre parce que ces souches comme l a fait remarquer Mme Quersin ne sont pas capables de causer des lesions au niveau des cultures de tissus employees habituellement

[1] Ol n G t W l n T A h Vir f s h 7 191 1957

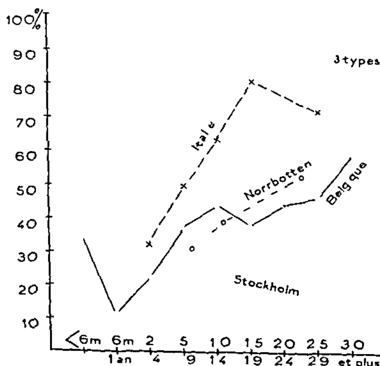


FIG 4 (*See text*)

(souches immunisantes mais non cytopathogenes)

Figure 4 montre les anticorps contre les 3 types de virus Comparaison de l etat d immunite de la population belge a celui de la Lombardie Italie et a celui de deux regions suedoises dont l une (Stockholm) connait generalement un nombre de cas beaucoup plus eleve que l autre (Comte de Norrbotten) [1] On voit que l immunite belge vaut a peu pres celle de la region suedoise la moins atteinte par la maladie

Figure 5 montre les anti orps contre le type 2 au moins encore une comparaison entre l etat d immunite de differents pays

BRAZIL

Dr Oswaldo P Campos

We have presented to the past conferen es statistics of infantile paralysis cases admitted to the Jesus Hospital in Rio de Janeiro In Brazil it has been our purpose to demonstrate that the disease constitutes a twofold problem First poliomyelitis is a very serious heath problem which has increased in importance each year and second it strikes in over 93 per cent of cases the 0 5 year age group thus being obviously an extremely difficult orthopedic problem

During the last 3 year period from June 1954 to May 1957 1 058 new cases of poliomyelitis were admitted to the Jesus Hospital which with the addition of 136 private cases brings the total to 1 194 which is the number of new victims of infantile paralysis brought to us for our personal observation

Table 1 shows that the pattern of the disease has not changed the 0 3 year age group being more prevalent with a percentage of 88 10

Of the 1 194 cases 146 were stricken before the age of 6 months reflecting a percentage of

Table I Age Incidence

Age in Years		Incidence (Percentage)
0–1	702 cases	58 79
1–3	350 cases	29 31
3–5	68 cases	5 69
5–10	33 cases	2 77
10 up	18 cases	1 50

See footnote on p 13

[2] Gio anardi A Monaci V et Bonetti F IIIe Symposium asso Europ Polio pp 47 54 1956

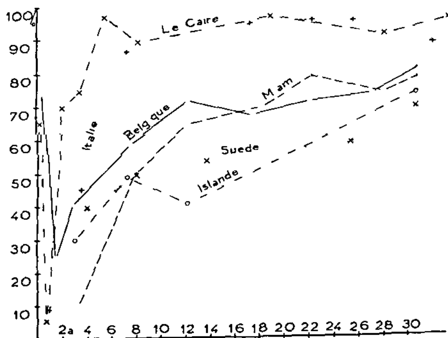


Fig 5 (*See text*)

12 22 which is a much higher rate than the 3 76 per cent reported by us in 1954[3]

These data only confirm what we have stressed previously that poliomyelitis infection due to very poor hygienic conditions still pre vailing in both large and small cities through out Brazil is widespread

The infantile type is highly prevalent Dr Mauricio Martins da Silva and Dr Jerome T Syverton[5] working along the same line as other investigators in many parts of the world recently have fully confirmed these clinical and epidemiologic assumptions Blood samples of 111 children living in Rio de Janeiro were sent to the University of Minnesota for serologic typing and specific polio antibody titer deter minations The 111 children submitted to this investigation were sele ted from 3 different socioeconomic levels

Group A consisted of 29 children taken from the slums or nearby densely populated rural areas where living conditions are very primitive and promiscuity a predominant characteristic

Group B consisted of 58 children taken from middle-class families living in small houses or apartments having running water and other hygienic facilities

Group C consisted of 24 children taken from the upper wealthier class It is noteworthy that according to the epidemiologic viewpoint there is not a complete separation of the slums in Rio de Janeiro The slums and the wealthy sections are both rather mixed in a very indiscriminate manner

The search for the presence and the titer of specific antibodies against polio viruses as deter mined by tissue cultures showed Group A to have antibodies against the 3 types of polio viruses in 79 per ent of the cases Group B in 53 per cent of the cases while Group C in only 29 per cent

The presence of antibodies against at least 1 of the 3 types of viruses was found in 100 per cent of Group A in 97 per cent of Group B and in 96 per cent of Group C the average age being 8 11 and 18 years respectively Thus it proves that latent immunity is acquired more rapidly in the poorer classes than in the groups better endowed economically Although no definite data are available the attack rates seems to diminish with increasing age among the poorer classes The classes forming Groups B and C of the Martins da Silva and Syverton investigations seem to be more susceptible to the disease although there is not any noticeable difference in age selection patterns among the 3 different groups We would like to call atten tion to the observations referred to in our report to the Second International Poliomyelitis Con ference showing that paralytic polio is a rarity among underprivileged children living in the slums of Rio which constitute the majority of Group A of the above mentioned authors During epidemic times however because of changes in virus virulence or in host conditions we have seen children with nutrition deficiency stricken by polio which otherwise seems to be a very uncommon occurrence

After the 1953 54 epidemic polio is certainly undergoing a transformation in the Federal Dis trict and the surrounding cities in the State of Rio de Janeiro it being definitely on the up ward trend and recent observations warrant the assertion that the same is true for many regions of the country

The admission rate of acute cases to the Jesus Hospital prior to the 1953 54 epidemic was 3 to 4 cases each month showing a flare up during the hot and damp summer season After the 1953 54 epidemic which was reviewed in our report to the Third International Poliomyelitis Conference[3] the monthly rate of admission to the Jesus Hospital stayed at the much higher level of an average of 2? cases as one can see in Table 2 as for example for the year of 1956

In 1956 there were 211 cases with sequelae admitted to our hospital However during that year many of those stricken came to the hospital only after the acute stage was over This brings the attack rate well over 10 per 100 000 of popu

TABLE 2 ADMISSION RATE OF ACUTE CASES TO JESUS HOSPITAL

January	8
February	11
March	12
April	20
May	16
June	21
July	32
August	47
September	39
October	33
November	22
December	14

lation and one can draw the conclusion that after the 1953-54 epidemic infantile paralysis reached an almost permanent epidemic level in the Federal District. It will be worthwhile to note that the 1953-54 epidemic of Rio de Janeiro with an incidence of 21.88 in each 100 000 of population according to Aristides Paes de Almeida[1] and J. G. Lacorte,[4] reached a higher level than the recent Argentine epidemic which showed the figure of 17.5 in each 100 000 inhabitants.

We would also like to mention the fact that the clinical manifestations of the disease have not changed much after that terrible epidemic, the spinobulbar and the encephalitic types still occurring with great frequency.

VACCINATION PROGRAM

In the latter part of 1956, with the mounting evidence as to the safety and the effectiveness of the Salk poliomyelitis vaccine and taking the opportunity of the export release of the vaccine by the American Government, a pioneering program of immunization was planned for the city of Rio de Janeiro.

In Brazil as in many parts of the world a lack of enthusiasm for the Salk vaccine still exists and some resistance to its use by parents and even doctors is encountered. This of course applies to any type of preventive vaccination which seldom is freely accepted by the general public. The decision to test the Salk vaccine in Rio de Janeiro was taken after Dr. Raphael de Souza Paiva, Director of the Department of Pediatrics in the Federal District and his assistants Drs. Alvaro Aguiar and Otavio da Veiga, returned from the 8th International Conference of Pediatrics held in Copenhagen in 1956. They were very much impressed by the papers presented on the subject during that conference. We did stress to Dr. Souza Paiva the necessity of such a trial on our return from the United States in April, 1956.

The different aspects of the problem were analyzed and the nature of poliomyelitis in Brazil was particularly taken into consideration on account of questions raised by some of our eminent specialists who based their objections on recommendations made in Stockholm by the World Health Organization in January 1956.[6] Finally an elaborated program was presented to Dr. Darcy Monteiro, Health Commissioner of the Federal District; this met with his enthusiastic approval and in November 1956 a vaccination trial was begun in Rio de Janeiro in a region where polio is very common, endemic and essentially infantile.

The first lot of vaccine was imported by the Municipal Government and because of legal technicalities was allotted only to the 16 different pediatric districts into which the metropolitan and urban areas of Rio de Janeiro are divided. In these health centers any child within the 6 month to 3 year age group, the group most commonly stricken, could be vaccinated free of charge. A special committee to work under Dr. Souza Paiva's direct supervision was appointed. This committee issued strict norms and regulations for the use of the vaccine and a well-organized system of control cards for necessary references and statistics was established. A special identification card is given to the family as a memento not only for the Salk vaccine but also for recording a complete polio vaccination program as well as any other vaccines given to the child up to the age of 5 years. This card in a simple manner gives educational information about the Salk vaccine, the idea being to secure the confidence of the parents and to free them from undue apprehensions.

The results of the first 3 months of the vaccination program in spite of the favorable publicity fell very short of the expectations. From November 13, 1956, to February 2, 1957 only 14 053 children were inoculated and what was very discouraging only 1 650 returned for the second inoculation, which was almost an anticipation of a complete failure of our program.

In February 1956 the situation was revaluated and it was decided that this age group should be extended up to 6 years. At this time an intensive campaign was directed to the general public through the press, the radio and television, which imparted information on the Salk vaccine stressing the necessity of the parents co-operating with the health authorities and warning the public against the possible rise of the incidence of polio in the months of March, April and May. This drive was very gratifying, raising the presentation for vaccination considerably, and by June 8, the number of first inoculations had reached 51,334.

Unfortunately the attendance for the second inoculation again fell far below a desirable figure since only 27,535 children showed up for this indispensable dose which is the insur ance of a good protection against paralytic polio

We are now calling on private organizations etc to other health services military hospitals so that co-operate in this vaccination program so that most of our infantile population which is esti mated to be of about 350 000 from 0 to 6 years can derive the benefits of the only weapon for fighting paralytic poliomyelitis at our disposal at the present time We realize that compulsory vaccination against polio for obvious reasons lies many years ahead

This pioneering program is also educational in scope It serves mainly to ward off the fear created about the poliomyelitis vaccine because of the few accidents which occurred during 1955 It is well known that thay were discov ered promptly and corrected to the point where it can be said that there is no other biologic product which is surrounded by as many safe guards and tests of safety as the Salk vaccine which is being distributed at the present time by the various manufacturers We are very happy to say that this goal has been unmistak ably attained which is proved by the fact that the Department of Health and Welfare (Secre taria Ceral de Saude e Assistencia) of Rio de Janeiro is now receiving daily from all o er Brazil requests for advice about the use of the vaccine I am sure that from now on no re sponsible practicing physician will assume the unforgivable responsibility of contraindicating the polio vaccine only to see his patient stricken a little later by this terrible disease as has hap pened in 3 tragic instances brought to my knowledge

Until now we have registered only 3 cases of paralytic polio after the vaccination and their histories follow in detail without unnecessary comment

Case 1 N A H f m le 1 year 3 months of age F rst dose of S lk vacc ne was admini tered on Jan ary 4 1957 On J nuary 12 temperatures of 103 and 104 w re record d sh was restless and shak ng of the head pers sted for 72 hours followed by com plete recovery On January 23 sh fell sick g in A t mpe at re of 10? w reported and the pat ent nd cat d that he l ft leg was extremely sens ti e to touch

This p cture lasted for about 3 days Later the pat ent was unable to walk because the left leg was flail Exam nat on re ealed slight atrophy of the left th gh and leg

Muscular examination showed the following

Gluteus maximus	Weak
B ceps	Trace
Tib alis Anticus	0
Tib al s posticus	0

Reflexes were as follows

Left patellar	0
Left achilles	0

Case 2 H J S male 11 months old First dose of Salk vacc ne was given on December 7 1956 the second dose on January 7 1957 On Ma ch 31 the patient was very sick and vomit ng with h gh tem perature nstipation and urine retent on On the th rd day there was flacc d paralys s of both lower limbs followed by paralys s of the upper extremities and marked invol ement of the resp ratory muscl s particularly the int rcostal group Spinal fluid cells 86 proteins 0 gr 15 per cent sodium chlor des 6 grs per cent glucosis 0 48 per cent The child reco ered f om the acute stage but there was exten ve residual paralys

Case 3 L P R male 5 years old First dose of Salk accine was gi en on Feb u ry 13 1957 the second on March 13 1957

On April 16 th boy had a h gh temperature with out any other particul symptoms A few days later he began to l mp and walked with the left foot turned nward Examination reveal d slight atrophy of the left leg he walks with the left foot slightly dropped and inverted The peron i and the tibiali anticus are ery weak The left peroneal reflex is absent

Fortunately the General Department of Health and Welfare in the Federal District is determined to carry on with this poliomyelitis vaccination program its extent being limited only by the present export restrictions of the United States It is also the desire of the Com mittee of this program to co-operate with the National Foundation for Infantile Paralysis in any investigations as to the effects of the vaccine in regions where poliomyelitis still strikes under its primitive aspects and where the children constantly exposed to the virus can develop a permanent natural immunity under the protec tion against paralytic polio by the vaccine

REFERENCES

1 Almeida A P Epidemiology of poliomye litis in Brazil Bol Inst. Pueri ultura 13 223 1956

2 Campos O P Poliomyelitis in Brazil *in* Poliomyelitis Papers and Discussions Pre



Fig 6 Cases of and deaths from poliomyelitis in the state capitals of Brazil in 1956

sented at the Second International Poliomyelitis Conference pp 140 142 Philadelphia Lippincott 1952
3 Campos O P Poliomyelitis in Brazil *in* Poliomyelitis Papers and Discussions Presented at the Third International Poliomyelitis Conference pp 34 35 Philadelphia Lippincott 1955
4 Lacorte J G Poliomyelitis in the Argentine Republic (private publication)
5 Martins da Silva M and Syverton J T Poliomyelitis survey in Rio de Janeiro Pub Health Rep 71 395 1956
6 La vaccination contre la poliomyelite revue preliminaire Chron Org mondiale de Sante 10 3 1956

Prof Jose Martinho da Rocha*

Though polio has long been known in Brazil scattered cases very often were not recognized in the acute phase and only belatedly with advanced paralysis were they taken to the state capitals for often little effective orthopedic treatment.

Prof Pinheiro Cintra of Sao Paulo was responsible for calling attention to the higher incidence of this illness in 1918 and for emphasizing the necessity of an early diagnosis. From then on Brazilian physicians became better acquainted with the disease which today is familiar to them and is more often correctly diagnosed The general impression is that in the past most cases were not reported Statistics are not enforced rigorously in Brazil s vast territory and therefore no exact data is available as to the extension of the disease in our country Once in a while more or less epidemic outbreaks occur in several cities of the interior in Rio de Janeiro and Sao Paulo The chief centers to receive polio victims are the wards of Prof Godoy Moreira in Sao Paulo and Achilles de Araujo in Rio de Janeiro apart from the orthopedic service of the Jesus Hospital of the Municipality of Rio which is under the supervision of Dr O Pinheiro Campos Based on the increasing number of cases in his own ward Campos at the Third International Conference in 1954 emphasized the fact that the disease is not as rare in this country as one is led to think Furthermore the incidence of encephalic and pontinobulbar cases has increased

The main epidemic outbreaks registered were those in Rio de Janeiro in 1939 (237 cases 23 deaths) in 1953 (746 cases 35 deaths) in 1956 (267 cases 13 deaths) and in Sao Paulo those of 1953 (136 cases) and 1956 (127 cases) The highest incidence registered was that of 1953 in Rio de Janeiro with 2 188 per 100 000 inhabitants (see Table 3) Figure 6 shows the number of polio cases and deaths (in parentheses) in Brazilian state capitals in 1956

Figures 7 10 by A Paz de Almeida† show the incidence in Rio de Janeiro in the past years with more accurate statistical data

There is no doubt that the disease most frequently strikes children under 5 years of age and that a slight or a more extensive paralysis is dominant

The Brazilian Foundation for Infantile Paralysis was founded in 1956 in the Institute of Puericulture of the University of Brazil by Prof Mauricio de Medeiros Minister of Health One of his first actions was to promote in November of 1956 a symposium on the epidemiology and the prophylaxis of polio Brazil s foremost sanitarians pediatricians orthopedists and research workers in virology took part in this symposium The following subjects were debated (1) Epi

* Official Delegate of Brazil to the Fourth International Poliomyelitis Conference Director of the Institute of Child Health of the University of Brazil (Rio de Janeiro)

† San t r n f om the H alth Ser ce of th Muni pality of R o de J n o

Table 3 Number of Clinical Cases in the State Capitals of Brazil*

Capitals	Years											
	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955
Manaus	—	—	—	1	—	—	—	—	1	2	—	3
Belém	—	—	—	1	1	5	1	6	—	3	—	2
Sao Luiz	—	—	—	—	—	3	—	3	2	—	—	1
Teresina	—	—	—	—	—	—	—	—	—	—	—	—
Fortaleza	6	1	1	1	12	9	—	9	2	3	3	1
Natal	—	—	—	—	1	—	—	1	—	1	—	—
Joao Pessoa	4	1	—	—	—	—	—	1	1	—	11	—
Recife	3	3	29	2	2	5	1	6	4	3	1	6
Maceió	2	—	4	1	—	9	1	—	3	—	1	1
Aracaju	—	—	1	—	1	—	1	—	1	—	—	—
Salvador	1	3	—	11	2	5	1	4	17	—	—	3
Vitória	—	3	1	4	1	2	—	—	5	—	1	1
Niterói	—	—	—	—	—	5	1	2	2	56	13	—
Rio de Janeiro	64	45	46	41	40	22	25	24	37	746	357	124
Sao Paulo	17	27	10	29	11	4	3	14	29	136	42	127
Curitiba	—	1	—	2	3	2	—	2	5	10	—	16
Florianópolis	1	11	7	3	3	5	5	14	—	3	1	—
Porto Alegre	7	10	68	5	27	68	15	12	15	19	18	—
Belo Horizonte	2	—	39	3	3	2	2	21	22	20	12	11
Goiânia	—	—	—	—	—	1	—	2	2	3	3	2
Cuiabá	—	18	1	—	—	—	—	—	1	—	—	—

St ti t c fr tl F l r l S of th Health Min try

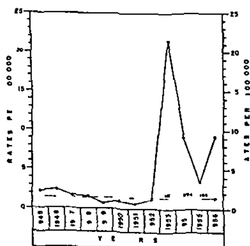


Fig 7 Annual rates per 100 000 population of notified cases (residents) in Rio de Janeiro

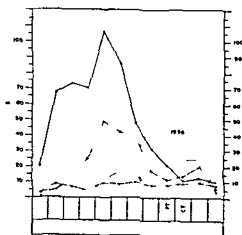


Fig. 8 Monthly distribution of cases (residents) in Rio de Janeiro.

demiology of Poliomyelitis in Brazil (Speaker A Paz de Almeida) (2) Evaluation of the Immunity Against Poliomyelitis (Speaker J Travassos) (3) Vaccine with Virus (Speaker Prof Paulo de Goes) (4) Active Immunity Against Polio and Salk Vaccine (Speaker J Madureira Para (5) Universal results of Anti poliomyelitis Vaccination (Speaker Prof J Martinho da Rocha)

The main conclusions reached were the following

1 Polio is a disease that occurs eventually or endemically in this country there are occasional epidemic outbreaks of minor or major extension

2 It is necessary to apply for an improvement of vital statistics on the epidemiology in order to afford better knowledge of the disease in our country

3 It is necessary to perform surveys concerning the epidemiology based on the serology of specific antibodies for poliomyelitis carried out with samples representative of the population concerning age and socioeconomic conditions

4 It is necessary to equip the laboratories in *order to enable them to identify the types of* viruses isolated in the epidemic areas

5 The available data show a variation in the specific age incidence with an overwhelming concentration of cases (paralysis) at low ages (80 per cent under 3 and 90 per cent under 5 years) as has already been recorded in certain areas

6 The frequency of extensive epidemic outbreaks recorded in certain areas (Rio de Janeiro Sao Paulo Niteroi and even smaller towns in the interior of some of the states) leads to the assumption that there is a possibility that we are on the way to an epidemization of polio in those areas

7 It is virtually impossible to immunize with the Salk vaccine all Brazilian children even if one restricted the age to 5 Importing the vaccine would be too expensive there is a lack of trained personnel to give inoculations 3 times a year and the classifying of the types of viruses has not yet been done nor serologic studies made of the susceptibility

Due to the tremendous repercussion in our press of the Argentine polio outbreak in 1956 the Secretary of Health of the Municipality of Rio de Janeiro has started vaccination of children and so far there have not been any accidents in that city Dr O Pinheiro Campos Brazilian delegate will discuss this subject

The most objective and accurate data about immunization of children in Rio was published in the *Public Health Reports* Vol 71 April 1956 based on the work of Drs M Martins da Silva and J T Syverton done at the University of Minnesota Their research gave the percent*age of antibodies in 111 sera of children in* Rio de Janeiro selected from 3 social groups Group A consisted of 29 children (average age 8) living in poor conditions and in unhygienic quarters with lack of drainage and running water or in collective quarters Group B was composed of 58 children (average age 11) from middle-class families living in modest houses or hygienic flats with running water and drainage with no crowding and with better economic standing Group C consisted of 24 children (average age 18) of the upper class

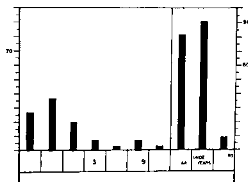


Fig 9 Percentage distribution of cases by age (residents) in Rio de Janeiro

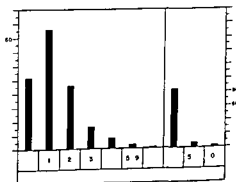


Fig 10 Specific case rates by age (residents) in Rio de Janeiro

TABLE 4 PERCENTAGE OF ANTIBODIES IN GROUPS A B C AND D AND E

GROUPS	NUMBER	AVERAGE AGE	PERCENTAGE WITH ANTIBODIES AGAINST			
			NO TYPE	1 TYPE	2 TYPES	3 TYPES
A	29	8 6	0	8 4	17 2	79 4
B	58	11 8	3 4	20 6	22 4	53 4
C	24	18 3	4 2	29 1	37 5	29 1
D	26	6 5	30 7	23 1	30 7	15 4
E	43	20–30	20 9	41 8	20 9	16 2

Table 4 shows the percentage of antibodies for the 3 groups as compared with Groups D and E of Minnesota U S A composed of sons of professors at the University or students The percentages were 100 per cent in Group A 97 per cent Croup B and 98 per cent in Group C

This work tends to demonstrate that a greater part of Rio s children especially in the low socioeconomic classes are naturally immune to polio

CANADA

DR F P NAGLER

The Canadian program of vaccination against poliomyelitis commenced in April 1955 as a co-operative project between the provincial departments of Health and the Department of National Health and Welfare the several departments equally sharing the costs The vaccine was produced by the Connaught Laboratories Toronto employing the Maitland technic and using the Mahoney the MEFI and the Saukett strains in the preparation of the trivalent vaccine Safety and potency tests similar to those in use in the U S A were carried out simultaneously by the manufacturers of the vaccine and by the Laboratory of Hygiene of the Department of National Health and Welfare in Ottawa Up to the present time about 60 lots of vaccine varying from 300 to 600 liters each have been submitted to the Department for testing Most of these lots and a limited number of lots submitted by American manufacturers have been released by the Department for general use

In 1955 about 800 000 Canadian children mostly between the ages of 5 and 9 years were inoculated with 2 to 3 doses while up to the end of March 1957 another 2 000 000 children of preschool and school age received at least 2 injections of the vaccine It is hoped that by the end of March 1958 all Canadians up to the age of 19 years (about 6 000 000) will have been vaccinated

Before evaluating the results of the Canadian vaccination program of 1955 and 1956 it is necessary to consider the incidence rate of poliomyelitis in these years in relation to the rate of previous years The rate of paralytic poliomyelitis in 1955 was 28 per cent of the average incidence rate for the 5 years (1950 1954) preceding while the rate in 1956 was 19 per cent of that average (1 900 cases/year 1950 1954) The low incidence rate of the last 2 years has made evaluation of the results of the vaccination program somewhat difficult since the numbers of reported cases in both the vaccinated and the unvaccinated groups were relatively small In 1955 for instance only 5 cases of paralytic polio were reported in 600 000 vaccinated children under observation and 51 cases in 890 000 unvaccinated children of the age group of 5 9 years In 1956 11 paralytic polio cases in a total of 1 860 000 vaccinated children and 136 cases in 2 140 000 unvaccinated children of similar age groups were reported in Canada

Considering the decline in the over all incidence rate of paralytic poliomyelitis in Canada during the past years it could be assumed that without vaccination this decrease would have occurred equally in all age groups However when calculating the expected incidence rate for 1956 it was noted that more cases than might have been expected occurred in the age group from 0-4 years while the calculated and the observed rates of the age groups of 20 years and over were comparable A consistently lower rate in observed cases as compared with the number of expected cases was found in the age

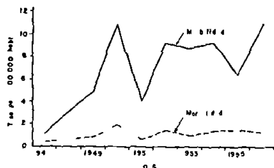


Fig 11 Morbilidad y mortalidad por poliomielitis en Chile 1947 1956

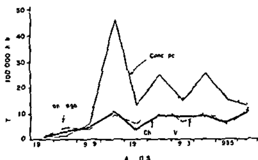


Fig 12 Morbilidad por poliomielitis en Valparaiso Santiago Concepcion y Chile 1947 1956

groups between 5 and 19 years Therefore it seems reasonable to assume that there has been a selective reduction of paralytic cases particularly in the school age groups since 1955 This reduction might well be a reflection of the protective effect of the Salk vaccine administered during the past 2 years It is proposed to follow changes in the age distribution pattern of paralytic poliomyelitis as they may occur in Canada during the forthcoming years

CHILE

Dr Hernan Romero

Las comunicaciones presentadas a las tres Conferencias anteriores (Nueva York 1948 Copenhague 1951 Roma 1954) nos han dado oportunidad para senalar el aumento progresivo de la poliomielitis en Chile que no ha hecho sino acelerarse ultimamente En efecto la tasa de morbilidad se ha multiplicado mas de 50 veces en este cuarto de siglo (0 2 × 100 000 en 1933 y 11 en 1956) Los empujes de verano tienen clara tendencia ascendente y en ellos se pueden distinguir cinco epidemias sobresalientes en 1943 44 1949 50 1951 52 1953 54 y 1955 56 Ordinariamente este prevalecer estacional no se ve claro en las tablas anuales porque nuestro año calendario comienza y termina en estio Las mayores incidencias se dan entre Octubre o Noviembre y Abril o Mayo con la cumbre en Diciembre y Enero Hasta hace poco las epidemias habian quedado circunscritas a las ciudades mayores Recientemente se las ha observado tambien en poblaciones de mediano tamaño y aun en pueblos rurales

Si bien la distribucion por edades sigue acusando desplazamiento todavia se observan las tasas mas altas en el segundo año de la vida el 80 de los casos en el primer trienio y el 90 % en el primer quinquenio Entre los ninos mayores y los adultos la enfermedad continua siendo manifiestamente excepcional Se nota predileccion por el sexo masculino La denuncia es siempre defectuosa y el 70 % de los pacientes corresponde a ninos hospitalizados frente a un 2 % de notificaciones provenientes de medicos particulares Se advierte mejor conocimiento de las formas no paraliticas y abortadas

El Instituto Bacteriologico dispone de laboratorio bien equipado que esta en manos de tecnicos competentes y experimentados Han logrado ellos el aislamiento y el cultivo de unas 218 cepas de virus de polio Su mayoria corresponde a los tres tipos antigenicos mejor conocidos y se distribuyen a su vez en un 80 % para el Brunhilde 15 % para Lansig y 5 % para Leon Uno que otro presenta variaciones Las epidemias han sido causadas en su gran mayoria por el tipo 1 Se ha encontrado frecuencia apreciable de virus Coxsackie y menor de ECHO que representa aproximadamente un 15 % de los hallazgos Los estudios serologicos indican que a los cinco años de edad mas del 50 % de los individuos tienen anticuerpos para uno o mas viruspolio En frecuencia decreciente corresponde a los tipos 2 1 y 3

Diversas partidas de vacuna Salk han sido importadas por empresas y personas particulares y su empleo no ha dado lugar a ningun contratiempo Ahora el Servicio Nacional de Salud se ha encargado de su importacion en escala mayor y la esta ofreciendo al publico en sus Centros de Salud y a precio de costo La entregará gratuitamente a los indigentes Además el Servicio

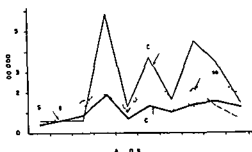


Fig 13 Mortalidad por poliomielitis en Valparaiso Santiago Concepcion y Chile 1947 1956

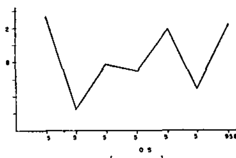


Fig 14 Morbilidad por poliomielitis en Chile 1950 1956

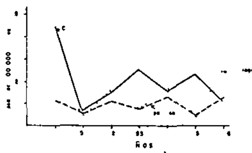


Fig 15 Morbilidad por poliomielitis en Santiago Valparaiso y Concepcion 1950 1956

practicará vacunaciones sistemáticas en una ciudad (Concepcion) con po o mas de 100 000 habitantes con el objeto de estudiar las diferencias entre grupos vacunados y no vacunados En atencion a la distribucion por edades observada está recomendando inmunizar a los ninos menores de cinco anos La existencia de problemas sanitarios de mayor envergadura no le permite todavia realizar esta empresa en gran escala Se está considerando seriamente la posibilidad de prepararla en Chile y tanto los estudios como las gestiones necesarios para el proposito estan avanzados

La feliz circunstancia de que el Servicio Nacional de Salud dispone de la gran mayoria de las camas hospitalarias del pais ha permitido disponer del numero requerido para los casos de esta enfermedad y dar facilidades especiales para su traslado Se cuenta con numero suficiente de aparatos de respiracion artificial En cambio las dotaciones para rehabilitacion y el personal respectivo son todavia insuficientes

Una ley está proxima a conceder fondos especiales para desarrollar la rehabilitacion Esta actividad quedará naturalmente incorporada en el Servicio Nacional de Salud pero los reglamentos respectivos son suficientemente elásticos como para permitir su administracion expedita Habrá departamentos de medicina fisica y rehabilitacion en todos los hospitales de 200 o más camas y uno o más institutos con dotacion mayor y fabricacion de aparatos ortopedicos La necesidad de desarrollar esta especialidad se deja sentir agudamente y constituye preocupacion preferente de algunas autoridades

Las caracteristicas epidemiologicas de la poliomielitis en Chile nos hacen suponer que la vacunacion pueda ser particularmente eficaz Nuestras colectividades estan perdiendo su inmunidad ante nuestros ojos y se podria suponer que estamos en el momento preciso en que se la puede estimular considerablemente con el empleo de un antigeno de eficiencia aun relativa Nuestra maquina administrativa permite llevar a la práctica una medida de esta especie y las ensenanzas que pueda aportar habrán de ser utiles para otros paises de condiciones similares

CZECHOSLOVAKIA

Dr Vilem Škovránek

EPIDEMIOLOGISCHE SITUATION

Polioepidemien treten in der CSR seit dem Jahr 1939 auf Seit dieser Zeit wiederholen sich Polioepidemien in Intervallen von 4 bis 5 Jahren Die durchschnittliche ganzstaatliche Morbidität erreicht in epidemischen Perioden ca 20 Kranke auf 100 000 Einwohner in ausserepidemischen Perioden 4 bis 8 Kranke auf 100 000 Einwohner (falls die ausserordentlich niedrige Morbidität

des Jahres 1955 nicht in Betracht gezogen wird) Die Entwicklung der Morbiditat vom Jahr 1939 bis zum Jahr 1956 demonstriert am besten die graphische Veranschaulichung auf der sehr gut die zwei letzten Epidemien (1948 und 1953) ersichtlich sind Die Morbiditat ist aber beim Auftreten von Epidemien nicht gleich massig auf das ganze Staatsgebiet verlagert sie unterscheidet sich grundlegend in verschiedenen Staatsgebieten wahrend die Morbiditat an *machen Orten die Ziffer von 50 bis 80 Kranken* auf 100 000 Einwohner uberschreitet ist sie an anderen Orten nur gering Dies demonstriert sehr gut wenigstens ein Kartogramm aus der Epidemie 1953 Was die Altersinzidenz anbelangt ist das Maximum der Krankenanzahl bisher bei Kindern im vorschulpflichtigen Alter wenn auch fortlaufend eine Verschiebung der Morbiditat auch in einige hohere Jahrgange zu beobachten ist Auf dem Graph der Altersinzidenz ist dies gut ersichtlich Dabei besteht ein gewisser Unterschied zwischen den westlichen *(Bohmen und Mahren) und ostlichen (Slowa*kei) Staatsgebieten was durch die abweichende historische und gesellschaftliche Entwicklung dieser Staatsgebiete erklart werden kann Die fortlaufende Verschiebung der Morbiditat in

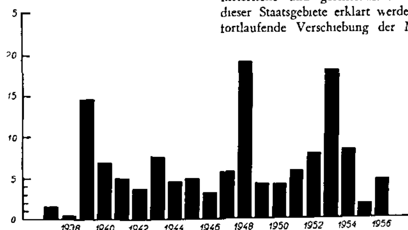


Fig 16 Poliomyelitis in Czechoslovakia 1937 1956 (notified cases per 100 000 inhabitants)



Fig 17 Poliomyelitis in Czechoslovakia in 1953 (per 100 000 inhabitants in different districts)

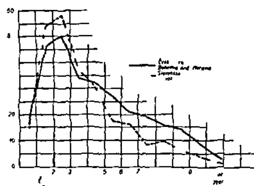


Fig 18 Age group morbidity (average of the years 1951 1955 per 100 000 inhabitants)

einige hoheren Altersgruppen demonstriert ein weiterer Graph auf dem zum Vergleich die Aenderungen in der Morbiditat vom Jahr 1937 bis zum Jahr 1952 angefuhrt sind Die epidemiologischen Analysen die in der CSR detailliert schon eine Reihe von Jahren durchgefuhrt werden vervollstandigen nunmehr auch ausfuhrliche virologische und immunologiche Analysen Die Altersinzidenz entspricht zu einem gewissen Masse auch den serologischen Befunden die vom Immunologischen Forschungsinsitut in Prag (Dr Zacek) durchgefuhrt wurden

KURZ UBER DIE HEILBETREUUNG VON ERKRANKTEN

In der CSR ist fur jeden Poliokranken eine unentgeltliche Heilbetreuung und Nachbehandlung seitens des Staates sichergestellt In jedem Kreis unseres Staates sind spezielle Heilzentren fur Polio errichtet ausserdem bestehen zwei gut eingerichtete Institute fur Nachbehandlung in Kurbadern (Johannisbad und Gross Lossin)

ZUR VAKZINATION GEGEN POLIO

Die epidemiologiche Situation in der CSR hat uns klar vor die Notwendigkeit gestellt die Erfahrungen amerikanischer wissenschaftlicher Fachleute fur eine Poliopravention auch in der

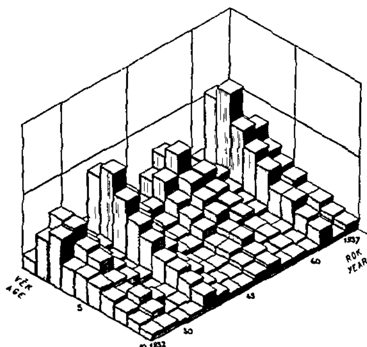
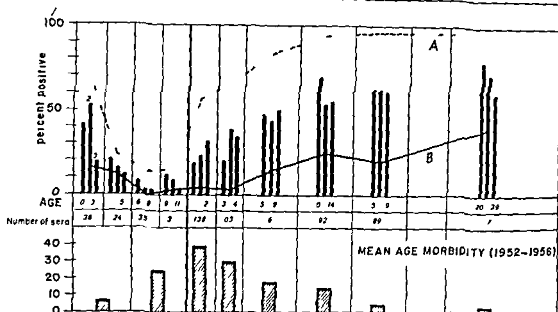


Fig 19 Age group morbidity in Bohemia and Moravia (per 100 000 inhabitants)

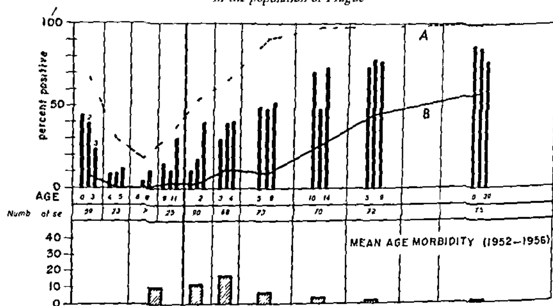


(778 examined sera were collected during first 3 months of 1957)

A Antibodies to any 1 type

B — Antibodies to all 3 types

Fig 20 Distribution of neutralizing antibodies against all 3 types of poliomyelitis viruses in the population of Prague



(602 examined sera were collected during first 4 months of (1957)

A Antibodies to any 1 type

B — Antibodies to all 3 types

Fig 21 Distribution of neutralizing antibodies against all 3 types of poliomyelitis viruses in the population of Jihlava (a rural district)

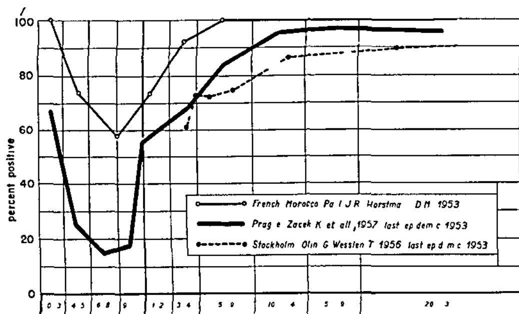


Fig 22 Neutralizing antibodies to any one type of poliomyelitis virus

500
400
300
200
100
0
100
0

1953
1954
1955
1956
Bohemia and Moravia
Slovakia

Fig 23 Poliomyelitis in Czechoslovakia 1953-1956

CSR auszunutzen Es wurden deshalb alle Vorbereitungen fur die Herstellung eines Poliompfstoffes noch in diesem Jahr getroffen Unsere ursprungliche Voraussetzung—Ende 1957 und zu Beginn 1958 zu impfen—wurde durch die atypische Poliomorbiditat im Jahr 1956 abgeandert Obwohl es zu keinem besonders hohen Auftreten kam war die Morbiditatsverschiebung bis in die spaten Jahresmonate verbluffend dies demonstriert gut ein Graph auf dem die Morbiditat vom Jahr 1953 bis zum Jahr 1956 veranschaulicht ist Ende des Jahres 1956 wurde deshalb im Ausland der Impfstoff bestellt und alle organisatorischen Massnahmen zur Einleitung der Impfaktion getroffen Infolge etwas verspateter Lieferung des Impfstoffes konnte mit der Aktion erst am 2 Mai 1957 begonnen werden wo sich die epidemiologische Situation im Vergleich mit dem Jahr 1956 schon sehr wesentlich verandert hatte Wir standen klar im Entfaltungsstadium einer Epidemie was gut aus einem Graph ersichtlich wo die Morbiditat nach Wochen angefuhrt ist (oben das Jahr 1956 unten das Jahr 1957) Trotzdem entschlossen wir uns zu impfen Wir erlauterten der Bevolkerung die Moglichkeit zeitlicher *Koinzidenz zwischen Impfung und eventueller* Erkrankung Ich kann hier erklaren dass unsere Bevolkerung die Impfaktion mit grossem Verstandnis aufgenommen hat ein Beleg dafur ist auch die hohe Impfbeteiligung Im Laufe der Monate Mai und Juni wurden [mit zwei Injektionen] insgesamt 2,318 074 Kinder geimpft davon mit zwei Injektionen 2,235 000 welche Zahl ungefahr 18 % der Gesamtbevolkerung der CSR darstellt und Kinder im Alter von 6 Monaten bis zu 7 Jahren in der Mehrzahl der Stadte noch Kinder bis zu 11 bzw bis zu 13 Jahren einschliesst

Zur Organisation der Impfaktion mochte ich anfuhren dass sie zentral vom Ministerium fur Gesundheitswesen in den Kreisen und Bezirken von den Kreis und Bezirkshygienikern geleitet wurde Mit der Durchfuhrung der Impfung waren Aerzte des normalen Netzes des Gesundheitsschutzes vor allem Kinderarzte betraut wobei bei der Impfung freiwillige Arbeiter des Tschechoslowakischen Roten Kreuzes behilflich waren Eine ausfuhrliche klinische und virologische Untersuchung jedes Falles von Erkrankungsverdachtes ist sichergestellt Bei der Impfaktion kam es zu keinen ernsteren Komplikationen Auf Grund danischer Erfahrungen wurde die Impfung mittels intrakutanner Technik durchgefuhrt verabfolgt wurden immer zwei Injektionen zu je 0 1 bis 0 15 ml also in jedem Fall durchschnittlich 0 25 ml Impfstoffes *Dies war einerseits durch einen gewissen* Mangel an Impfstoff bedingt andererseits durch den Umstand dass bei der hohen gesundheitlichen Reife unserer Bevolkerung nur schwerlich eine bloss experimentelle Impfung

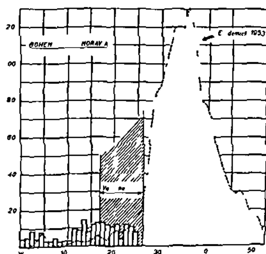


Fig 24 Cases of paralytic poliomyelitis in Bohemia and Moravia in 1957

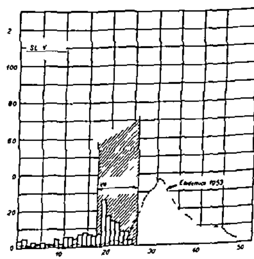


Fig 25 Cases of paralytic poliomyelitis in Slovakia in 1957

von beschrankten Kindergruppen hatte erklart werden konnen Ausserdem haben wir voraus gesetzt dass durch den positiven Einfluss einer Massenimpfung d i durch kollektive Immunitat gewisse Mangel in de individuellen Immunitat uberbruckt werden konnen

Die Ergebnisse der Aktion, die erst am 1 Juli d J abgeschlossen wurde konnen begreiflicher weise noch nicht bewertet werden konnen Ich kann nur die Schnelligkeit der Aktion bewerten die von unseren Aerzten wirklich vorbildlich durchgefuhrt wurde

Die Entwicklung der Morbiditat wahrend der Aktion ist aber trotzdem interessant sodass ich mir schliesslich doch gestatte sie zu demonstrieren

Sollten sich unsere Voraussetzungen uber den Einfluss der kollektiven Immunitat bewahrheiten konnte unsere umfangreiche und zeitlich rasch durchgefuhrte Aktion in den nachsten Monaten und Jahren die epidemiologische Poliosituation der CSR beeinflussen

DENMARK

Dr E Juel Henningsen

Since the 2 severe outbreaks of polio in 1952 and 1953 comprising more than 3 000 paralytic cases which I described at the Third International Poliomyelitis Conference in 1954 Denmark has been spared epidemic polio Only sporadic cases have occurred there were 72 cases in 1954 24 in 1955 and 37 in 1956

The after treatment and the rehabilitation of the many patients with paralyses have placed a great responsibility on the public hospital system the Danish National Association for Infantile Paralysis and the Society and Home for Cripples

In Blegdamshospital Copenhagen 70 patients with respiratory pareses are still hospitalized A special institution for these chronic patients is being built A few patients have been moved to their own homes and given complete practical and financial support from private and public authorities

The great event in the fight against poliomyelitis in Denmark has been the introduction of polio vaccination When Salk published his first studies on polio vaccine in 1952 the Danish State Serum Institute (Statens Seruminstitut) was prepared to take up experimental work on this line The scientists in the Institute had followed closely the work in Salk s laboratory After the Third International Polio Conference in Rome in September 1954 it was decided to start a Danish polio vaccine production The polio vaccine was manufactured at the Danish State Serum Institute and was prepared according to Salk s method No other vaccine has been used in Denmark

On April 12 1955 when Dr Thomas Francis Jr published the report Evaluation of 1954 Field Trial of Poliomyelitis Vaccine the Danish State Serum Institute had vaccine in stock for about 400 000 children The National Health Service decided that 400 000 school children from the first 5 grades 7 to 12 years of age should be the first to be vaccinated

After careful consideration the following principles for vaccination were established

1 The vaccination should be entirely voluntary and permission obtained from the parents before vaccination

2 The children were to be given 3 injections —the first and the second with about a 4 week interval the third about 12 months later

3 At each injection the child should be given 2 intradermal injections in the forearm the total amount of vaccine employed being about 0.3 ml

The vaccination of these 400 000 school children was started on April 20 1955 not more than a week after the publication of the American report

The program was organized by the country s 66 medical officers of health in collaboration with the local branches of the Danish Medical Association About 600 school physicians and specially appointed general practitioners carried through the campaign which included more than 98 per cent of the school children in the first 5 classes in 4 000 schools in Denmark The campaign was completed without any accidents.

A special antipoliomyelitis vaccination act was passed by Parliament on May 24 1955 In consequence of this act vaccination is voluntary and free of charge for everybody under 40 years. Vaccination is performed by *all general practitioners* who receive their fees from the State This is in accordance with the general principles followed in Denmark for prophylactic health measures.

The amount of vaccine ready for use in April

1955 was not sufficient for immediate vaccination of the 2 700 000 inhabitants under 40 years of age The Minister of the Interior in accordance with the amount of vaccine available has decided when the different age groups should be allowed to be vaccinated Children under school age were vaccinated in the fall of 1955 in the spring of 1956 all persons up to 25 years of age were permitted vaccination In June 1956 all persons under 40 years of age were allowed vaccination

To what extent has the population made use of this service of the Government of Denmark?

Ninety nine per cent of children between 9 months and 14 years of age have been vaccinated against polio and no difference between numbers of children vaccinated in the capital the provincial towns and the rural areas has been observed

Ninety three per cent in the age group 15 to 19 and 85 per cent between 20 to 39 years have been vaccinated and again there is no difference between the capital and the provincial towns though in the rural areas the figures are slightly smaller It can be stated moreover that no difference between rich and poor was found

Since September 1956 anyone over 40 years of age may be vaccinated at his own request as expected only relatively few have asked for vaccination

The figures which are increasing daily show *that nearly 100 per cent of the Danish population* in the age groups especially exposed to polio are vaccinated More than 2,500 000 out of a population of 4 500 000 have already benefited from the polio vaccination

In order to follow the antibody response blood specimens from about 1 500 school children were taken immediately before the first injection and about 1 month after The serologic results have shown that the vaccination has had the anticipated effect

As I mentioned earlier Denmark has had only few sporadic cases of poliomyelitis during the last 3 years I want to stress that we have not drawn any conclusions from this as to the effect of the vaccine In this connection it must be remembered that the high percentage of vaccinations in the population means that there is no control group in the scientific sense of the word

Denmark has had no real vaccine surplus during these years However it has been possible to make vaccine available to Iceland as well as to Norway In these two countries 400 000 persons have been vaccinated with Danish vaccine

You may recall that with the background of the big poliomyelitis epidemics in Norway and Denmark a Scandinavian Poliomyelitis Board was appointed in 1953 The collaboration among the Scandinavian countries has resulted in many valuable negotiations through these years and practical assistance was given to Iceland during a severe outbreak of poliomyelitis in 1955

On behalf of the Scandinavian Poliomyelitis Board and on behalf of my own country I have the honor to greet the Fourth International Poliomyelitis Conference and to express our gratitude to our Swiss hosts and to the National Foundation for Infantile Paralysis

EGYPT

Prof M Diwany

In Egypt poliomyelitis is not a new disease Ancient Egyptian sculptors have left us a relief dating from the 18th Dynasty (about 1500 B C) in which the priest Ruma is shown with an atrophic shortened leg and a paralytic pointed foot suggesting that the man obviously had suffered from spinal poliomyelitis

In spite of this historical evidence poliomyelitis was considered to be a rare and interesting disease at the time when I first joined the University Children s Hospital at Cairo in 1931 Since then poliomyelitis has shown a progressive increase in incidence and a change from a sporadic occurrence to an endemic type spread In the year 1939 for example the number of cases referred to the Electrotherapy Department *of our Hospital was only 38* whereas in 1953 there were 889 cases i e more than a twenty threefold increase in a period of 15 years In 1955 there were 1 130 cases and in 1956 757 cases If it is considered on a very modest scale that an equal number is also treated in other hospitals and private clinics th total incidence of polio in 1953 can be assumed as exceeding 1 800 cases making a case rate of 9 per 100 000 The average case rate in the U S A for 1932 to 1946 is 7.26 per 100 000

TABLE 5 SEASONAL INCIDENCE OF DIFFERENT TYPES OF POLIO

	1955												1956	
TOTAL No	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	FEB
	63	67	48	75	106	133	86	112	173	176	126	94	115	59
Type 1	NST	1	0	0	2	7	10	5	NST	0	3	11	4	1
Type 2	NST	2	1	2	3	13	16	1	NST	4	2	3	2	0
Type 3	NST	2	1	0	0	4	4	3	NST	1	1	2	0	1

NST = N [illegible]

In Egypt poliomyelitis has not broken loose from its age limitation to early childhood. Most of the cases are under 2 years of age, the highest peak being between 10 and 16 months. Males are affected more than females in the proportion of 3 to 2. A seasonal variation in incidence is not striking—cases are encountered throughout the year with some monthly fluctuation. The spinal form of the disease is the commonest form met. Lower limbs are affected more frequently than upper limbs and both upper and lower extremities more than the trunk. The incidence of bulbar polio is less than 1 per cent in contrast to more than 6 per cent abroad. This may be explained by the earlier age incidence in our country and the fact that tonsillectomy usually is performed after the third year of life. There has been observed among our infants and children a high incidence of isolated 7th nerve paralysis which is probably of polio virus etiology.

The work of Paul *et al* carried out in Egypt in 1950, 1951 and 1952 throws much light on the question of age incidence. Neutralizing antibodies to the Lansing, the Leon and the Brunhilde types were found to be acquired at an early age among Egyptians. With improvement of hygienic and social standards we expect the disease to affect older age groups and to approach the pattern observed today in the U S A and in Europe.

Polio virus studies were made in our department in collaboration with the United States Naval Medical Research Unit No. 3 under the supervision of Dr J. Taylor, now Professor of Virology at Yale University, and with the help of Dr D. Horstmann, Associate Professor of Virology at Yale University.

In the series studied, specimens for isolation of the virus were taken only from patients in the first 14 days of the illness. Monkey kidney or Hela cells were used for isolation, but for typing only monkey kidney cells were used. It was found that Type 2 exceeded both Types 1 and 3 in incidence, giving a total incidence of 44 per cent. Types 1 and 3 gave an incidence of 39 per cent and 17 per cent respectively. It was also observed that the domination of Type 2 (Type 2 48.6 of cases, Type 1 35.4, Type 3 16) was more marked during the hot months, while Type 1 (Type 1 63.3 of cases, Type 2 23.3, Type 3 13.3) was more frequent during the cooler months (Table 5). As regards the age incidence, the youngest child from whom the polio virus was isolated was $3\frac{1}{2}$ months old and the oldest was 5 years old. Type 1 was predominant below $2\frac{1}{2}$ years, after which the other types prevailed.

The 100 per cent isolations in this series were almost reached at $2\frac{1}{2}$ years for Type 1, at 4 years for Type 2 and at 5 years for Type 3. The most extensive paralysis was found in patients infected with Type 2 virus, the extent of paralysis with the other 2 types being about equal (Table 6).

Compared with the U S A, Holland and South Africa, where Type 1 is strictly predominant, in Egypt Type 2 exceeds both Type 1 and Type 3 (Table 7). According to Paul *et al* in the isolations that were made in Egypt in 1943, 3 out of 7 strains were Type 2 polio virus, i.e., 40 per cent. Also serologic studies done by Paul in Egypt in 1950 among normal infants and children revealed that Type 1 polio virus antibodies lag behind Type 2 antibodies.

TABLE 6 RELATION BETWEEN TYPE OF VIRUS ISOLATED AND EXTENT OF PARALYSIS

TYPE	1 EXTREMITY	2 EXTREMITIES	2 EXTREMITIES AND TRUNK OR 3 EXTREMITIES	TRUNK AND 4 EXTREMITIES (AND DIAPHRAGM)
1 (47 cases)	37 (79%)	8 (15%)	1	1
2 (53 cases)	31 (59%)	15 (30%)	4	3
3 (22 cases)	17 (77%)	4 (19%)	0	1

TABLE 7 PREDOMINANCE OF TYPE 2 VIRUS IN EGYPT AS COMPARED WITH OTHER COUNTRIES

TYPE	EGYPT (1955)	U S A (1953)	HOLLAND (1952)	SOUTH AFRICA (1954)
1	39%	80%	68%	42%
2	44%	6%	28%	33%
3	17%	14%	4%	23%

In Egypt the Coxsackie virus is quite common and in some instances may be associated with viremia *It has been accidentally isolated* from blood at the United States Naval Medical Research Unit No 3 on 19 occasions and frequently was isolated from stools oropharyngeal secretions and flies Types B-2 B-4 A 2 A 3 A 4 A-6 and A 8 were isolated from Egyptian children

It is important to mention that ECHO virus Prototype 1 was isolated in 1954 from an Egyptian child in Cairo by Dr J Melnick The child was apparently normal and *asymptomatic* Dr Horstmann examined rectal swabs from 200 asymptomatic Egyptian children and out of these 96 i e 48 per cent yielded orphan viruses In a previous work done in 19?? Dr Horstmann found that out of 300 children examined 126 isolations were orphans i e 4? per cent

Could the orphan viruses by being spread so widely in our community among our children *in an asymptomatic fashion or with only minor* symptoms be cross immunizing us against some other more vicious viruses in the same way that the West Nile virus is rendering us cross immunized to some of the other encephalitides viruses?

Should we ignore a virus simply because it is associated with a mild ailment or even none? Mutation may convert a relatively harmless organism into something capable of causing a lethal infection

Probably there is a relation between viruses of different types and the appearance of paralysis in some cases when no neurotropic virus was isolated indicates that under certain circumstances a comparatively benign organism may somehow acquire unseen destructiveness It is possible that a person with either passive or active immunity to the 3 conventional types of polio may still be susceptible to a poliolike illness paralytic or nonparalytic And conversely *a person infected with* any of these viruses may be mistakenly believed to be immune to polio

FINLAND

DR M E PARMALA

In Finland so far poliomyelitis *has not taken* the form of an alarming epidemic In 1945 1954 and 1956 the worst years in this respect *the number of reported* clinical cases amounted to about 18 to 20 per 100 000 inhabitants However it has been discovered in laboratory tests for poliomyelitis neutralizing antibodies that the mean immunity is 50 70 and 90 per cent at the ages of 5 20 and 40 respectively Subclinical infection thus is fairly common

About 75 to 80 per cent of the reported clinical cases involve paralysis and about 12 to

15 per cent of these patients suffer from respiratory paralysis

Paralytic cases are attended to at the epidemic wards of rural communal hospitals or at home or in the bigger towns at special hospitals for communicable diseases Only the latter have the necessary facilities for giving rehabilitation treatment at an early stage If it proves impossible to arrange such treatment and if orthopedic surgery is required the patient is transferred to the State Invalid Hospital or to the Orthopedic Hospitals or the Rehabilitation Institutes run by private foundations and organizations As these hospitals also treat other patients who are in need of orthopedic treatment or rehabilitation the number of places available for polio cases at present is limited To meet the needs of northern Finland the building of a new State-owned invalid hospital is under consideration

The treatment of respiratory paralysis to the largest possible extent has been concentrated in the 3 largest hospitals for communicable diseases in the cities of Helsinki Turku and Tampere These hospitals have trained staffs and the necessary respirators at their disposal In addition a center containing 6 to 7 beds is now being equipped at the Provincial Hospital of the town of Oulu to cater to acute cases in the north of Finland For patients suffering from chronic respiratory paralysis (the present number in Finland is about 20) the State center with 12 to 15 beds will be ready in the near future Since the treatment of respiratory paralysis incurs great expenses—annually some 3 to 5 million marks per patient—individual communities have been unable to cover hospital expenses from their funds Such costs also being beyond private means the necessary financial assistance has been applied for from the State funds In the beginning of 1957 a new law went into effect according to which the State pays all acceptable expenses incurred from the treatment of respiratory paralysis

The transportation of the above mentioned polio patients is a difficult problem in Finland The majority of the population lives in the southern and the southwestern parts of the country whereas the eastern and the northern parts are populated sparsely and the network of roads is insufficient Since cases of respiratory paralysis call for urgent transportation it is as a rule necessary to use air transport. Our large waterways offer suitable landing places for sea planes and in the winter when the lakes are icebound planes provided with skis can land easily But during the spring thaw as well as in the autumn before freezing air transport cannot be used Therefore the purchasing of a sufficient number of helicopters appropriate for such transport is under consideration

Another difficulty arises from the shortage of fully trained nursing staffs As the number of hospital nurses is inadequate in general and as the nursing of patients suffering from respiratory paralysis is especially strenuous both physically and mentally the fully trained personnel prefers easier employment The situation may become easier in this respect as there are good hopes that the gap in the nursing profession will be gradually filled in during the course of the next few years

Vaccination against polio has been going on since 1954 The first vaccinations were of an experimental character and they were performed on a small scale in certain districts earmarked for this purpose As of spring 1957 mass vaccination involving about 150 000 children was started throughout the country The object is to carry on vaccination and to increase the number of children eligible for it so that finally all age groups which are susceptible to polio will be included in this scheme

FRANCE

Dr. Lacombre

Au cours des 3 annees qui ont suivi le precedent Congres la frequence de la poliomyelite n a pas marque de variations notables en France

Si la maladie y marque depuis 1917 date à laquelle fut instituee l obligation de sa declaration une progression continue celle-ci reste relativement lente Rappelons que la moyenne annuelle des cas declares de 700 pour la periode 1917 1929 est passee à 500 pour la periode 1930-1942 et a 1 400 pour la periode 1943 à 1953 l indice moyen de morbidite pour cette derniere periode étant de 3 4

1950 reste l annee ou fut enregistree la plus forte poussee de poliomyélite en France avec 1 979 cas declares et un indice de morbidité s elevant a 4 7

La France garde en ce qui concerne la polio-

myélite une situation assez privilégiée et cela même par rapport à la plupart des pays limitrophes de son territoire.

Le tableau 8 comporte l'indication des totaux annuels de cas déclarés et des indices annuels de morbidité enregistrés en France depuis 1954.

TABLEAU 8. LES TOTAUX ANNUELS DE CAS DÉCLARÉS ET LES INDICES ANNUELS DE MORBIDITÉ ENREGISTRÉS DEPUIS 1954

ANNÉES	NOMBRE DE CAS	INDICES DE MORBIDITÉ RAPPORTÉS 100 000 HABITANTS
1954	1 534	3,5
1955	1 834	4,2
1956	1 150	2,6

A noter que les déclarations concernent essentiellement des formes paralytiques.

Calculés à partir de ces chiffres, le nombre moyen de cas déclarés au cours de ces 3 années qui s'élève à 1 500 et l'indice moyen de morbidité qui est de 3,4 apparaissent très voisins de ceux enregistrés pour la période antérieure.

Il convient toutefois de signaler une certaine recrudescence de la poliomyélite au cours des premiers mois de 1957 (651 cas déclarés pour les 25 premières semaines contre 259 pour la période correspondante de 1956, mais le total de 1956 était le plus bas enregistré depuis 1948).

L'étude de la répartition des cas par âge montre toujours une prédominance très marquée de la maladie dans les tranches d'âge inférieures: au cours des 3 années en cause 36 % des cas sont survenus de 0 à 4 ans, 31 % de 5 à 14 ans et 33 % au dessus de 15 ans, taux sensiblement comparables à ceux observés pour la période précédente (35 %, 28 % et 37 %).

En ce qui concerne la répartition des cas par sexe, l'androtropisme de la maladie reste net. Pour les 3 années en cause 57 % des cas ont été masculins contre 43 % de cas féminins, taux identiques à ceux observés pendant la période précédente.

Si la morbidité poliomyélitique n'a pas marqué dans ces dernières années de variations notables on note par contre une diminution de la mortalité.

En effet, alors que pour la période 1943-1953 le nombre moyen annuel des décès était de 236, l'indice moyen de mortalité (nombre de décès par poliomyélite pour 100 000 habitants) de 0,6 et le taux de léthalité (nombre de décès pour 100 cas de la maladie) de 16, pour la période 1954-1956 le nombre moyen annuel de décès n'est que de 146, l'indice moyen de mortalité de 0,3 et le taux de léthalité de 9,6.

TABLEAU 9. L'INDICE DE MORTALITÉ 1954-1956

ANNÉES	NOMBRE DE DÉCÈS DÉCLARÉS	INDICE DE MORTALITÉ	TAUX DE LÉTHALITÉ
1954	141	0,3	9,1
1955	184	0,4	10,0
1956	114	0,27	9,9

Il semble qu'on soit en droit d'établir un rapport entre cette régression de la mortalité et les efforts faits pour améliorer les conditions de traitement des formes aiguës de la maladie et notamment des formes respiratoires.

Le nombre des décès reste plus élevé dans le sexe masculin que dans le sexe féminin et les chances de survie sont plus limitées à l'âge adulte que dans l'enfance.

La maladie garde en France une allure endémo-sporadique avec une recrudescence saisonnière.

L'augmentation de la durée de la poussée saisonnière annuelle, déjà signalée lors du précédent Congrès, s'est précisée encore dans les 3 dernières années, la recrudescence annuelle se prolongeant maintenant pendant 7 mois. Cette poussée saisonnière se manifeste de façon plus intense dans certaines zones de localisation géographique très variable d'une année à l'autre.

Ainsi en 1954 c'est le département du Morbihan en Bretagne qui fut le plus atteint, en 1955 un département du Sud-Ouest, la Corrèze, et en 1956 trois départments méridionaux: l'Ariège, l'Hérault et la Haute-Garonne. L'extension de la maladie dans la moitié sud du territoire déjà indiquée dans le rapport précédent s'est donc confirmée au cours des 3 années écoulées.

En vue de tenter d'approfondir les connaissances sur l'épidémiologie de la poliomyélite des recherches immunologiques sont poursuivies en France depuis plusieurs années. Elles ont été entreprises en premier lieu par le Laboratoire des Virus de l'Institut Pasteur de Paris.

Depuis 1954 en outre le Laboratoire de Virologie de la Societe d Etudes et de Soins pour les enfants poliomyélitiques (S E S E P) poursuit egalement des recherches de cet ordre avec l aide de l Institut National d Hygiene

A signaler egalement les examens pratiques par le Laboratoire d Hygiene de la Faculté de Medecine de Lyon avec l aide de l Institut National d Hygiene et de la Caisse Nationale de Securite Sociale

Ces travaux ont pour objectifs essentiels

1 De chercher à determiner la courbe d acquisition des anticorps en fonction de l age dans certains echantillons de la population française

Une enquete a ete menée à cet égard par l Institut Pasteur de Paris à partir des serums preleves chez 357 enfants de 20 mois à 21 ans confies au Service de l Assistance Publique de Paris (enfants d un milieu social modeste)

L autre est effectuee par le Laboratoire de la *Societe d Etudes et de Soins pour les enfants* poliomyelitiques à partir des serums preleves chez 546 enfants hospitalises dans les villes de Tours le Havre Rouen et Nantes

Une troisième enquete qui a déjà porte sur plus de 700 serums est poursuivie par le Laboratoire d Hygiène de la Faculté de Médecine de Lyon *Les sérums examines proviennent d une* part d enfants originaires de toutes les regions de la France et groupes dans une meme collectivite et d autre part d enfants vivant en milieu rural

Les resultats obtenus à le suite des 2 premières enquetes sont sensiblement comparables

(A) *Entre* 2 ans et 2½ ans dans les 2 groupes examines 50 des enfants possedent au moins 1 des 3 types d anticorps

(B) Dans le groupe d age 7—9 ans l Institut Pasteur trouve 40 des sujets possedant les 3 types d anticorps Dans le groupe d age de 7 ans 50 des enfants sont trouves porteurs de 3 types d anticorps par le Laboratoire de la Societe d Etudes et de Soins pour les enfants poliomyélitiques

(C) Entre 19 et 21 ans 77 des sujets examinés par le Laboratoire de l Institut Pasteur possedent les 3 types d anticorps

Les enfants de 15 ans examines par le Laboratoire de la Société d Etudes et de Soins pour les enfants poliomyélitiques presentent dans la proportion de 70 des anticorps aux 3 types de virus

Les resultats obtenus par le Laboratoire d Hygiene de la Faculte de Medecine de Lyon s averent un peu differents des precédents Pour les memes catégories d age la proportion des sujets porteurs des 3 types d anticorps est inferieure a celles observees precedemment (1) dans le groupe d age de 5 a 9 ans 14 seulement des sujets seraient porteurs des 3 types d anticorps (2) dans le groupe d age de 15 a 19 ans si tous les sujets presentent des anticorps 43 seulement sont porteurs des 3 types d anticorps

Mais il apparait que les sujets examines appartiennent a des milieux sociaux economiques differents de ceux des enquetes precedentes

2 De tenter de preciser les variations de l acquisition des types d anticorps en fonction du lieu et du temps Les recherches effectuees à cet egard par le Laboratoire de la Societé *d Etudes et de Soins pour les enfants poliomye*litiques ont amene a constater qu entre les annees 1954 et 1955 se manifestait pour le meme groupe d age et dans des agglomerations differentes une regression du pourcentage des porteurs d anticorps du Groupe 2 alors qu augmentait celui des porteurs du Type 3 Ce re*sultat a d ailleurs ete confirme par les isolements* de virus effectues tant par l Institut Pasteur que par le Laboratoire de la S E S E P à partir des matieres fecales de poliomyelitiques ou de sujets contacts

Si entre 1954 et 1955 une predominance constante du Type 1 a continue a etre observee en France on a pu constater aussi une pro-

TABLEAU 10 TYPES ANTIGENIQUES DES DIFFERENTES SOUCHES DE VIRUS ISOLÉES A PARTIR DES SELLES DE MALADES POLIOMYELITIQUES HOSPITALISÉS

	Type 1	Type 2	Type 3
Annee 1954			
Résultats, Institut Pasteur	39 %	50 %	9 %
Résultats S E S E P	70 %	21 %	9 %
Année 1955			
Résultats Institut Pasteur	72 %	[illegible]	1[illegible] %
Résultats S E S E P	65 %	9 %	6 %

myelite une situation assez privilegiee et cela meme par rapport a la plupart des pays limitrophes de son territoire

Le tableau 8 comporte l indication des totaux annuels de cas declares et des indices annuels de morbidite enregistres en France depuis 1954

TABLEAU 8 LES TOTAUX ANNUELS DE CAS DECLARES ET LES INDICES ANNUELS DE MORBIDITE ENREGISTRES DEPUIS 1954

ANNÉES	NOMBRE DE CAS	INDICES DE MORBIDITE RAPPORTES 100 000 HABITANTS
1954	1 534	3 5
1955	1 834	4 2
1956	1 150	2 6

A noter que les declarations concernent essentiellement des formes paralytiques

Calcules a partir de ces chiffres le nombre moyen de cas declares au cours de ces 3 annees qui s eleve a 1 500 et l indice moyen de morbidite qui est de 3 4 apparaissent tres voisins de ceux enregistres pour la periode anterieure

Il convient toutefois de signaler une certaine recrudescence de la poliomyelite au cours des premiers mois de 1957 (651 cas declares pour les 25 premieres semaines contre 259 pour la periode correspondante de 1956 mais le total de 1956 etait le plus bas enregistre depuis 1948)

L etude de la repartition des cas par age montre toujours une predominance tres marquee de la maladie dans les tranches d age inferieures au cours des 3 annees en cause 36 % des cas sont survenus de 0 à 4 ans 31 % de 5 a 14 ans et 33 % au dessus de 15 ans taux sensiblement comparables a ceux observes pour la periode precedente (35 % 28 % et 37 %)

En ce qui concerne la repartition des cas par sexe l androtropisme de la maladie reste net Pour les 3 annees en cause 57 % des cas ont ete masculins contre 43 % de cas feminins taux identiques a ceux observes pendant la periode precedente

Si la morbidite poliomyelitique n a pas marque dans ces dernieres annees de variations notables on note par contre une diminution de la mortalite

En effet alors que pour la periode 1943 1953 le nombre moyen annuel des deces etait de 236 l indice moyen de mortalite (nombre de deces par poliomyelite pour 100 000 habitants) de 0 6 et le taux de lethalite (nombre de deces pour 100 cas de la maladie) de 16 pour la periode 1954 1956 le nombre moyen annuel de deces n est que de 146 l indice moyen de mortalite de 0 3 et le taux de lethalite de 9 6

TABLEAU 9 L INDICE DE MORTALITE 1954 1956

ANNÉES	NOMBRE DE DÉCÈS DECLARÉS	INDICE DE MORTALITE	TAUX DE LÉTHALITE
1954	141	0 3	9 1
1955	184	0 4	10 0
1956	114	0 27	9 9

Il semble qu on soit en droit d etablir un rapport entre cette regression de la mortalite et les efforts faits pour ameliorer les conditions de traitement des formes aigues de la maladie et notamment des formes respiratoires

Le nombre des deces reste plus eleve dans le sexe masculin que dans le sexe feminin et les chances de survie sont plus limitees à l age adulte que dans l enfance

La maladie garde en France une allure endemo-sporadique avec une recrudescence saisonniere

L augmentation de la duree de la poussee saisonniere annuelle deja signalee lors du precedent Congres s est precisee encore dans les 3 dernieres annees la recrudescence annuelle se prolongeant maintenant pendant 7 mois Cette poussee saisonniere se manifeste de façon plus intense dans certaines zones d localisation geographique tres variable d une annee a l autre

Ainsi en 1954 c est le departement du Morbihan en Bretagne qui fut le plus atteint en 1955 un département du Sud Ouest la Corrèze et en 1956 trois departments méridionaux l Ariège l Herault et la Haute-Garonne L extension de la maladie dans la moitie sud du territoire déjà indiquee dans le rapport précedent s est donc confirmee au cours des 3 annees ecoulees

En vue de tenter d approfondir les connaissances sur l epidemiologie de la poliomyélite des recherches immunologiques sont poursuivies en France depuis plusieurs annees Elles ont éte entreprises en premier lieu par le Laboratoire des Virus de l Institut Pasteur de Paris

Depuis 1954 en outre le Laboratoire de Virologie de la Societe d Etudes et de Soins pour les enfants poliomyelitiques (S E S E P) poursuit également des recherches de cet ordre avec l aide de l Institut National d Hygiene

A signaler également les examens pratiques par le Laboratoire d Hygiene de la Faculté de Medecine de Lyon avec l aide de l Institut National d Hygiene et de la Caisse Nationale de Securite Sociale

Ces travaux ont pour objectifs essentiels

1 De chercher a determiner la courbe d acquisition des anticorps en fonction de l age dans certains echantillons de la population française

Une enquete a eté menee a cet egard par l Institut Pasteur de Paris à partir des serums preleves chez 357 enfants de 20 mois à 21 ans confiés au Service de l Assistance Publique de Paris (enfants d un milieu social modeste)

L autre est effectuee par le Laboratoire de la Societe d Etudes et de Soins pour les enfants poliomyelitiques à partir des sérums preleves chez 546 enfants hospitalises dans les villes de Tours le Havre Rouen et Nantes

Une troisième enquete qui a deja porte sur plus de 200 serums est poursuivie par le Laboratoire d Hygiene de la Faculte de Medecine de Lyon Les serums examinés proviennent d une part d enfants originaires de toutes les regions de la France et groupes dans une meme collectivité et d autre part d enfants vivant en milieu rural

Les resultats obtenus à le suite des 2 premieres enquetes sont sensiblement comparables

(A) Fntre 2 ans et 2½ ans dans les 2 groupes examinés 50 des enfants possedent au moins 1 des 3 types d anticorps

(B) Dans le groupe d age 7—9 ans l Institut Pasteur trouve 40 des sujets possedant les 3 types d anticorps Dans le groupe d age de 7 ans 50 des enfants sont trouves porteurs de 3 types d anticorps par le Laboratoire de la Societe d Etudes et de Soins pour les enfants poliomyelitiques

(C) Fntre 19 et 21 ans — des sujets examinés par le Laboratoire de l Institut Pasteur possedent les 3 types d anticorps

Les enfants de 15 ans examinés par le Laboratoire de la Societé l Etudes et de Soins pour les enfants poliomyelitiques présentent dans la proportion de 70 des anticorps aux 3 types de virus

Les resultats obtenus par le Laboratoire d Hygiene de la Faculte de Medecine de Lyon s averent un peu differents des precedents Pour les memes categories d age la proportion des sujets porteurs des 3 types d anticorps est inferieure a celles observees precedemment (1) dans le groupe d age de 5 a 9 ans 14 seulement des sujets seraient porteurs des 3 types d anticorps (2) dans le groupe d age de 15 à 19 ans si tous les sujets presentent des anticorps 43 seulement sont porteurs des 3 types d anticorps

Mais il apparait que les sujets examines appartiennent à des milieux sociaux economiques differents de ceux des enquetes precedentes

2 De tenter de preciser les variations de l acquisition des types d anticorps en fonction du lieu et du temps Les recherches effectuées a cet egard par le Laboratoire de la Societé d Etudes et de Soins pour les enfants poliomyélitiques ont amene à constater qu entre les annees 1954 et 1955 se manifestait pour le meme groupe d age et dans des agglomerations differentes une regression du pourcentage des porteurs d anticorps du Groupe 2 alors qu augmentait celui des porteurs du Type 3 Ce resultat a d ailleurs ete confirme par les isolements de virus effectues tant par l Institut Pasteur que par le Laboratoire de la S E S E P a partir des matieres fecales de poliomyelitiques ou de sujets contacts

Si entre 1954 et 1955 une predominance constante du Type 1 a continue à etre observee en France on a pu constater aussi une pro-

Tableau 10 Types Antigeniques des Differentes Souches de Virus Isolées à Partir des Selles de Malades Poliomyelitiques Hospitalises

	Type 1	Type 2	Type 3
Année 1954			
Résultat Institut Pasteur	39%	50%	9%
Résultats S E S E P	70%	21%	9%
Année 1955			
Résultats Institut Pasteur	7[illegible]%	[illegible]%	1%
Résultats S E S E P	65%	9%	

gression sensible du Type 3 s effectuant surtout *aux detriments du Type 2*

Sur la base de ces resultats obtenus a partir de prelevements provenant de sujets originaires d agglomerations differentes il est permis d avancer que l evolution des 3 types de virus poliomyelitiques se fait vraisemblablement par poussees successives de grande diffusion

Bien que la poliomyelite ne constitue pas pour la France *ainsi qu on l a vu un probleme sani* taire de premier plan des efforts importants ont cependant ete faits au cours des dernieres annees afin de mettre a la disposition de la population les methodes therapeutiques les plus actuelles

1 L equipement du pays en centres de traitement des formes aigues de la maladie et specialement des formes respiratoires a ete complete

Etant donne d une part l incidence relative*ment faible de la maladie en France et d autre* part les conditions tres particulieres (tant en ce qui concerne le personnel que le materiel) qui s imposent pour le fonctionnement de centres de traitement des formes respiratoires de la poliomyelite il est apparu necessaire de ne les creer qu au sein des principaux centres hospitaliers regionaux

Treize centres specialises répartis sur l ensemble du territoire ont ainsi ete amenages Ils ont ete dotes du personnel et du materiel spe*cialises necessaires*

Afin d assurer la formation du personnel medical et infirmier affecte a ces centres des stages ont ete organises chaque annee dans les 2 centres de Paris les premiers mis en service

Des dispositions ont ete prises en outre pour que chaque centre regional puisse disposer des differentes varietes d appareils respirateurs connus poumons d acier et appareils assurant la respiration par voie endotracheale

Chaque centre regional dessert une circon*scription* geographique determinee et possede une ambulance qui assure le transport des malades de leur domicile au centre un medecin et une *infirmiere affectés a celle-ci peuvent* s il y a lieu dispenser les soins de premiere urgence

2 Dans le domaine de la reeducation fonctionnelle des infirmes poliomyelitiques le developpement des possibilit s de reeducation notamment dans les hopitaux publics se poursuit

Pendant cette periode enfin en France comme dans la plupart des autres pays le probleme de la prophylaxie de la maladie par la vaccination a ete l objet d une attention tres particuliere

Un vaccin français inactive a ete mis au point par l Institut Pasteur de Paris Les souches entrant dans sa composition ont ete selectionnees a partir de 270 souches isolees en *France Une* des caracteristiques essentielles du vaccin français est l utilisation pour sa preparation de souches *apathogenes des 3 types de virus*

Ce vaccin doit etre administré par voie sous cutanee la vaccination comportant 3 injections auxquelles il est conseille d adjoindre l annee suivante une injection de rappel

La vente du vaccin a ete autorisee depuis le 1er Juin 1956 sur presentation d une ordonnance medicale

La vaccination qui garde un caractere stricte ment facultatif peut-etre pratiquee soit au domicile du medecin praticien soit dans des centres *regionaux publics repartis dans les principales* villes du territoire

Dans ces centres actuellement au nombre de 15 la vaccination est effectuee gratuitement Elle est precedee et suivie de prelevements sanguins pratiques dans le but de determiner la presence la progression et la persistance des anticorps les examens necessaires etant executes dans le Laboratoire de Virologie auquel chaque centre de vaccination doit etre rattache

Cette methode est apparue comme celle susceptible *de fournir en France* les meilleures indications sur la valeur de la vaccination L incidence de la maladie y est en effet trop faible pour *qu on puisse obtenir des resultats sta* tistiquement valables par la comparaison de la morbidite de groupes de sujets vaccin s et de groupes non vaccines Plus de 100 000 vaccinations ont ete pratiquees maintenant en France

GERMANY

BUNDESREPUBLIK DEUTSCHLAND (WEST)

Dr Habernal

STAND

Fur das Gebiet der Bundesrepublik Deutschland gewinnt die Poliomyelitis seit d m I *Weltkrieg zunehmende* Bedeutung Die wellenformige Kurve der Erkrankungen an Kinderlahmung mit den typischen Spatsommergipfeln zeigt im Laufe der Jahrzehnte in Abstanden

von 4 5 Jahren hohere Erhebungen Die grosste Epidemie ereignete sich im Jahre 1952 mit einer Morbiditat von 1 96 je 10 000 Einwohner 1956 war ein sogenanntes Normaljahr mit einer Morbiditat von 0 79 je 10 000 Lebende Auffallend war dabei eine Verschiebung der hochsten Krankheitsziffern in den Monat Oktober

ALTERSVERTEILUNG

Bei einer breiten Streuung der Erkrankungen bis in das Erwachsenenalter liegt der hochste Anteil der Erkrankungen mit Lahmungen im 2 3 und 4 Lebensjahr Untersuchungen zur Aufstellung eines Antikorperkatasters ergaben dass die Kinder im 2 und 3 Lebensjahr gegen alle 3 Typen nur in wenigen Fallen und nur in durchschnittlich 50 gegen einen der Typen Antikorper besitzen Die Zahl der Kinder mit Antikorpern nimmt aber mit zunehmendem Alter rasch zu Bei den Untersuchungen der Zehnjahrigen wurden in 80 90 Antikorper festgestellt

VERHALTNIS DER ERKRANKUNGEN MIT UND OHNE LAHMUNGEN

Über das Verhaltnis der Erkrankungen mit und ohne Lahmungen seit 1953 geben die namentlichen Meldungen der Kinderlahmungsfalle an das Bundesgesundheitsamt Auskunft Auf 100 diagnostisch gesicherte Erkrankungen entfallen 70 75 Erkrankungen mit Lahmungen

Die Letalitat seit 1953 schwankte zwischen 5 1 und 7 8 /

BEKAMPFUNG DER POLIOMYELITIS

Zur Vereinheitlichung der Bekampfungsmassnahmen wurde 1954 die Deutsche Vereinigung zur Bekampfung der Kinderlahmung gegrundet In 8 Ausschussen bearbeitet sie die wissenschaftlichen Probleme der Poliomyelitis (Durch ihre Merkblatter und durch eine Wanderausstellung bemuht sie sich um die Aufklarung der Bevolkerung In arztlichen Zeitschriften publiziert sie Richtlinien und Zusammenfassungen uber den wissenschaftlichen Stand der Kinderlahmungsfragen)

Fur die Behandlung der akuten Kinderlahmung sind 29 Behandlungszentren meist in Kinderkliniken entstanden Sie dienen vor allen Dingen der Behandlung atemgestorter Poliomyelitiskranker und sind eingerichtet nach den Richtlinien der Vereinigung zur Bekampfung der Kinderlahmung

Fur den Transport in die Behandlungszentren stehen Spezialwagen des Deutschen Roten Kreuzes zur Verfugung Diese Wagen sind mit Atemgerat und Absaugegerat ausgestattet und mit geschultem Personal besetzt

Seit dem 1 April 1957 ist ein neues Korperbehindertengesetz in Kraft Dieses gibt auch fur die Nachbehandlung und Wiedereingliederung der infolge Kinderlahmung Korperbehinderten die arztliche wirtschaftliche und soziale Grundlage Es sichert die orthopadische Überwachung die Kostenregelung fur Heilverfahren und orthopadische Hilfsmittel und umfasst auch die Herstellung der Erwerbsfahigkeit und Berufsforderung

Nach Abschluss des akuten Erkrankungsstadiums und Entlassung der Erkrankten aus den behandelnden Kliniken und Krankenhausern werden die Nachbehandlung und Nachbetreuung durch den Landesarzt gemass den Bestimmungen und Moglichkeiten des Korperbehindertengesetzes geregelt Dieses Nachbehandlung erfolgt in besonderen orthopadischen Kliniken und orthopadischen Kinderkliniken Daneben stehen auch verschiedene Thermalbader zur Verfugung die sich speziell auf die Nachbehandlung der Kinderlahmung eingestellt haben

IMPFUNGEN

Zur Durchfuhrung von Schutzimpfungen gegen Kinderlahmung wurden im April diesen Jahres 1300 l Impfstoff aus U S A und 150 l aus Belgien eingefuhrt Die Impfungen wurden anfangs im wesentlichen beschrankt auf Kinder des 2 und 3 Lebensjahres Die Impfung ist freiwillig In einigen Landern der Bundesrepublik ist sie vollig kostenlos in anderen tragt die offentliche Hand nur bei wirtschaftlicher Bedurftigkeit der Eltern die gesamten Impfkosten sonst muss der Impfstoff von den Eltern bezahlt werden Die Impfungen werden unter Aufsicht der Gesundheitsamter durchgefuhrt Die Impfbeteiligung schwankte zwischen 20 und 80 der genannten Jahrgange Im ganzen wurden ca 450 000 Erstimpfungen durchgefuhrt. Eine Kontrolle und Auswertung der Impfergebnisse erfolgt durch das Bundesgesundheitsamt.

Ab Herbst 1957 wird voraussichtlich deutscher staatlich geprufter Impfstoff der Behringwerke zur *Verfugung stehen*

DEUTSCHEN DEMOKRATISCHEN REPUBLIK (EAST)

Dr A Kukowka

In der Deutschen Demokratischen Republik ist die Zahl der Polioerkrankungen auch in den letzten Jahren gering gewesen und ging seit 1954 zuruck

	Morbiditat (auf 100 000 E)	Letalitat
1954	6 58	11 38%
1955	5 72	9 00%
1956	3 85	7 40%

Alle Poliofalle und Verdachtsfalle werden in Poliomyelitiszentren fur akute Falle deren Zahl 20 betragt hospitalisiert und von Spezialarzten behandelt Die Beatmungsabteilung in diesen Zentren muss mit allen notwendigen *vielseitigen Apparaturen und Instrumentarien* in ausreichender Anzahl sowie mit dem erforderlichen speziell ausgebildeten Personal ausgestattet sein Nach Ablauf der vorgeschriebenen Isolierung und Behandlung werden die Kranken durchschnittlich nach 6 Wochen in die nach modernen Gesichtspunkten eingerichteten Poliomyelitis Rehabilitationszentren verlegt Die Zahl dieser Zentren betragt sechs Eines davon befindet sich in einem Thermalbad *Die Gesamtdauer der Behandlung betragt bis zu* drei Jahren und langer und zwar auf Kosten der Sozialversicherung bzw der Staatlichen Fursorge

Fur die Behandlung von Atemlahmungen fur akute Falle stehen in den "Poliomyelitiszentren fur akute Falle etwa 120 Tank Respiratoren (Eiserne Lungen) moderner Bauart sowie Apparaturen zur Durchfuhrung der intratrachealen Beatmung gemass der Lassen Methode zur Verfugung *Bei spinalen Atemlahmungen wird die* Verwendung von Tank Respiratoren (Eisernen Lungen) bevorzugt Bei bulbaren Atemlahmungen ist die intratracheale Beatmung die Methode der Wahl wahrend bei Schlucklahmungen Lagerungswechsel exakte Aspirationen usw sowie die Anwendung des selbstatig wirkenden Speichelsaugschlauchs nach Denecke empfohlen wird Es wird prinzipiell nicht allein auf die Auswahl der jeweils optimal geeigneten Apparatur sondern vor allem auf die Qualifikation des Beatmers Wert gelegt Bei *allen Beatmungsapparaturen soll grundsatzlich* neben dem positiven auch der negative Druck berucksichtigt werden Neben laufenden Kreislaufuntersuchungen usw wird speziell auf die Kontrolle der CO Ausatmungsluft mit ein wandfrei funktionierenden Apparaturen (Uras M Firma Hartmann & Braun Frankfurt a Main Infrared Analyzer Beckmann evtl Spezial-CO Messgerat Firma Drager Lubeck) Wert gelegt pH Messungen im Blut mittels *der elektrometrischen Methode konnen vorlaufig* nicht als vollig exakt anerkannt werden

Zwecks schneller Erfassung und Betreuung von *Atemlahmungsfallen sollen motorisierte* Teams ausgestattet mit transportablen Beatmungsapparaturen und allem sonst notwendigen Instrumentarium zum Einsatz kommen

Fur die Behandlung der Poliofalle speziell der Atemlahmungen werden geeignete Aerzte (einschliesslich HNO Aerzten und Anaesthesisten) ausgesucht bzw *in Spezialkursen und* in wiederholten Fortbildungslehrgangen herangebildet ebenso Krankenschwestern Pfleger und Mechaniker *fur die Beatmungsapparaturen*

Neben einigen kleinen Firmen widmet sich eine leistungsfahige Fabrik fur medizinische Apparate der Produktion von Tank Respiratoren (Eisernen Lungen) und von Beatmungsgeraten fur intratracheale Atemlahmungen Ein Arbeitskreis von Spezialingenieuren und medizinischen Fachleuten berat regelmassig die einschlagigen Firmen Es wird angestrebt auf dem Gebiete der Beatmungsapparaturen moglichst einfach zu bedienende dabei *solide und* gemass dem Stande der Wissenschaft hochwertige Apparaturen herzustellen

Ein modernes Institut fur Poliovirenforschung und Immunbiologie ist eingerich et und hat seine Arbeit begonnen

Als beratende Organisation auf dem Geb t der Polioprobleme steht dem Ministerium fur Gesundheitswesen das Poliomyelitis Komitee zur Seite dem Fachleute aus allen Disziplinen angehoren Das Poliomyelitis Komitee *steht im* regen Gedankenaustausch mit den entsprechenden Organisationen vieler anderer Lander

Poliompfungen haben im Bereich der Deutschen Demokratischen Republik bisher nicht stattgefunden nicht etwa infolge eines grundsatzlich ablehnenden Standpunktes viel mehr deshalb um zunachst die weitere Ent wicklung des Gesamtproblems abzuwarten

GREAT BRITAIN

ENGLAND AND WALES

Dr W H Bradley

I recall that at the second conference in this series Dr Christopher Andrewes mentioned cer tain gibes at Great Britain because her previ ously low incidence of poliomyelitis would place her among the less advanced countries He added Unfortunately we have gone up in the world since then

In this respect our first rise to eminence was in 1947 Now in the first part of 1957 the incidence of polio was higher than ever before the order of magnitude at the 23rd week being

1957—1 082 uncorrected notifications
1950— 840 uncorrected notifications
1953— 766 uncorrected notifications

The figures which follow relate to England and Wales which is a fair sample of the expe rience of Great Britain The population of Eng land and Wales is 44 667 000 and since the last conference the corrected notification rates of acute poliomyelitis (including polioencephalitis) both paralytic and nonparalytic have been

1954— 4 4 per 100 000
1955—17 7 per 100 000
1956— 7 2 per 100 000

The deaths numbered 134 270 and 137 respec tively

The heavy incidence in 1955 was exceeded only by 1947 and 1950 our peak years The year 1956 fell into a more normal group with the years 1951 to 1954 inclusive but the seasonal curve was unusual with a second peak in the 41st week followed by a hangover into 1957 at a considerably higher level than usual This has been inflated further by the occurrence of 3 or 4 discrete off season outbreaks which deserve special study and also call attention to the diffi culties of forecasting the incidence of poliomye litis

Steady progress is being made toward meet ing the ever increasing demand for diagnostic facilities Isolation of polio virus is now prac ticed in 10 of the Public Health Laboratories where in 1956 over 800 strains of polio virus were isolated equivalent to almost one third of reported cases Eighty seven per cent of these strains were Type 1 7 per cent were Type 2 and 6 per cent were Type 3[8] and investigations into the distribution of disease producing virus are now proceeding against a background of anti body studies which have given a complicated picture For example in a remote rural district where the disease had never been reported titers by tissue-culture neutralization test were as high as in the great city of Liverpool and infection with all 3 types of virus apparently had taken place recently in both areas[3]

In the large urban area of Southend 70 per cent of children under 5 had no polio anti bodies but in Belfast 80 per cent of children of the same age had antibody to all 3 types of virus (Editorial 1956) It was under these cir cumstances at Belfast that Professor G W A Dick attempted and discontinued the use of a live attenuated vaccine

Regarding formalized vaccine at the time of the Francis report in April 1955 manufacture of a vaccine incorporating the same 3 strains used in the U S A had already begun But later in the autumn of 1955 it was decided to substitute a Brunenders Type I strain for the Mahoney strain previously used and early in 1956 a vaccination program for children born in the years 1947 to 1954 was announced It was anticipated that the demand for vaccination would greatly exceed the supplies of vaccine which could be produced in the limited time available before the onset of the 1956 epidemic season and the opportunity was taken of issu ing the vaccine in such a way as to allow its protective value to be scientifically assessed There was enough vaccine only for 200 000 of the 1 900 000 children who registered for vac cination and by giving vaccine only to children born in certain designated months it was pos sible to accept those who registered but received no vaccine as strictly comparable controls It so happened that the number of children vac cinated was about the same as in the placebo controlled study in America in 1954 There was not a high incidence of poliomyelitis during the observation period with the result that the number of cases were small However protec

tion of the same order as was observed in the American trial has now been demonstrated and is quite substantial over the wider age range of 1½ to 9½ years. Vaccination with 2 doses administered at monthly intervals intramuscularly into the left arm is now proceeding at the rate of about 500 000 inoculations a month in the children of this age group and it is intended to continue immunization throughout the poliomyelitis season. There appears to be no doubt that the Brunenders strain incorporated in the British vaccine has conferred protection against the Type I infections prevalent in the country. The antigenicity of the vaccine to all 3 types was in the same range as that obtained with American vaccines[6]. A second manufacturer is now coming into production and a rapid improvement in the supply position can be expected.

This trial, like the Francis trial, revealed that the incidence of illnesses reported as nonparalytic poliomyelitis was uninfluenced by vaccination and it is instructive that whereas virus was *isolated from about 70 per cent of the specimens* examined in cases reported as paralytic, in the nonparalytic group the virus was isolated from only 26 of the 89 specimens obtained. The pathogenesis of aseptic meningitis reported as poliomyelitis but due to other causes remains a problem and is the subject of several investigations in the United Kingdom[1]. In the past 3 years the ratio of nonparalytic to paralytic notifications has been 1954 32.7 per cent, 1955 41.4 per cent, 1956 46.3 per cent. These percentages are to some degree a measure of the frequency of disease simulating poliomyelitis and consequently the calculated fatality from polio must be interpreted with caution. Nevertheless, it is gratifying to notice that mortality ratios for paralytic cases alone in 1955 and 1956 were 7.3 and 8.0 respectively, showing a distinct improvement on the previous decade when the corresponding figures had been as high as 14.2 per 100 notifications. This improvement could be due to a change in the character of the disease or to more accurate observation of minor degrees of paralysis but it may also be the result of better understanding of the treatment of patients with severe respiratory disease and the establishment of many special units for this purpose.

Reports on the provoking effects of certain inoculations first appeared in the English journals in 1950. A Committee of our Medical Research Council has reported approximate estimates of the risks associated with individual prophylactics[5], the average being 1 in 37 000 inoculations in the years 1951 to 1953 and steps are being taken to control this hazard. The provoking effect of tonsillectomies, recent or remote, has also been confirmed in a controlled study[4].

In addition to our comprehensive national health and welfare services which provide the basic needs of those stricken with poliomyelitis there are 3 voluntary bodies devoted entirely to their succor: the Infantile Paralysis Fellowship, the National Fund for Poliomyelitis Research and a co-ordinating United Kingdom Committee for Poliomyelitis established in 1956.

REFERENCES

1. Bradley, W. H.: Europ. Assoc. Poliomyelitis (in press), 1957.
2. Dick, G. W. A., and Dane, D. S.: British M. J. 1: 70, 1957.
3. Fallon, R. J.: Lancet 1: 65, 1956.
4. Knowelden, J.: Europ. Assoc. Poliomyelitis, vol. 3, p. 169, 1956.
5. Medical Research Council: Lancet 2: 1223, 1956.
6. British M. J. 1: 366, 1957.
7. British M. J. 1: 1271, 1957.
8. Ministry of Health: Annual Report C.M.O., London, Her Majesty's Stat. Off., 1956.

HUNGARY

PROF. GEORGE IVANOVICS

The incidence rate of poliomyelitis has increased somewhat in Hungary recently. The morbidity rate was 17.1 in 1943 and 13.9 in 1956 per 100 000 population. Another feature of poliomyelitis endemics in this country is the change of distribution of paralytic cases by age groups during this decade. A gradual decrease from 70 per cent to 64.4 per cent of the proportion of age group 0 to 4 years occurred between 1950 and 1952. On the other hand there was an increase in the proportion of this age group since 1953 (73.2 %), the trend of which reached its maximum of 81.1 per cent by 1954 and 1955[5].

Poliomyelitis strains isolated from paralytic cases and typed during the last few years are shown in Table 11

TABLE 11 DISTRIBUTION OF TYPES OF POLIOMYELITIS STRAINS ISOLATED IN HUNGARY BETWEEN 1954 1956

YEAR	NO OF STRAINS	DISTRIBUTION OF STRAINS TYPES 1	2	3	AUTHOR
1954	10	4	5	1	Pinter *et al*[6]
1954	9	3	6	0	Foldes[2]
1955	31	6	10	15	Molnár and Fornosi[4]
1956	8	3	4	1	Beladi and Szollosy[1]
Total	58	16	25	17	

The distribution of strains by their types is rather uncommon Type 2 strain dominated the recent endemics in Hungary The strains isolated during the last 3 years were recovered from patients residing all over the country

A study has been carried out by Pinter *et al*[6] *regarding the distribution of antibodies to different types of virus in our population* (Table 12) Healthy inhabitants from the city of Szeged with no poliomyelitis history in their past were bled in 1954 Their serum samples were tested for the presence of neutralizing antibodies in cultures made of human fetal tissues

The distribution of antibodies found in the inhabitants of Szeged in 1954 suggests that non manifest infections with Types 2 and 3 might have been relatively rare previous to virus isolations in Hungary The appearance and the rise of Type 2 antibody lags markedly behind Type 1 antibody A similar tendency somewhat less pronounced is apparent in the case of Type 3 antibody The low frequency of antibody to Types 2 and 3 viruses in early ages might *furnish a basis for elucidating the high* proportion of Types 2 and 3 strains isolated recently The neutralization test results namely the strikingly low proportion of antibody to Types 2 and 3 in the early years of life might involve a direct connection to the shift of incidence rate of disease toward very young ages in recent years

This report was closed in April therefore it does not deal with the severe outbreak which occurred quite recently in Hungary

REFERENCES

1 Béládi I and Szollosy E. Personal com*munication*
2 Foldes P Orvosi Hetilap 39 1078 1956
3 Ivanovics G Poliomyelitis Papers and Discussions Presented at the Third International Conference Philadelphia Lippincott 1955
4 Molnar E and Fornosi F Acta microbiol Acad Sc Hungary (In press)
5 Petrilla A Personal communication
6 Pinter M Ibraham E and Rávnay M Acta microbiol Acad Sc Hungary 3 399 1956
7 Statisztikai Havi Kozlemenyek No 1 and No 2 Budapest 1957

INDONESIA

DR SOEMIATNO

Before World War II poliomyelitis had been known to occur in Indonesia only very sporadically The first case was diagnosed at Menado (Island of Sulawesi) in 1931

It was in 1948 that multiple cases of poliomyelitis were reported *from Pladju (near Palembang Island of Sumatra)* These cases occurred among European children of the employees of the B P M (Dutch Oil Company) Almost at

TABLE 12 DISTRIBUTION OF NEUTRALIZING ANTIBODIES AGAINST THE 3 TYPES OF POLIOMYELITIS VIRUS IN HEALTHY PERSONS[6]

AGE GROUP	NO OF SUBJECTS	TYPE 1 POSITIVE	TYPE 1 PER CENT	TYPE 2 POSITIVE	TYPE 2 PER CENT	TYPE 3 POSITIVE	TYPE 3 PER CENT
½–2 years	21	6	28	2	9	4	19
3–7 years	29	17	59	13	45	16	55
8–15 years	39	23	59	29	74	21	54
16–30 years	37	27	73	30	81	25	67

100 000 and other areas had small local epidemics The most interesting event took place in Cork In a population of approximately 114 000 102 cases of paralytic poliomyelitis occurred—a rate of 89 per 100 000 The epidemic began early in July and was virtually over in October at any rate so far as paralytic cases were concerned This was the first epidemic of poliomyelitis recorded for this city Newspaper publicity getting the upper hand of reason magnified the event to the city's great financial loss The brunt of the epidemic in the city and the suburbs fell on infants and young children but some adult cases did occur and these were *generally more severe* *Some weeks after the* commencement of the epidemic in the city small groupings of cases occurred in the surrounding country within a radius of about 60 miles This spread took place in the country areas centered commercially on the city and the spread of the disease was neither as dramatic nor as rapid as in the city area These foci began in late July and persisted into February of this year Generally speaking the age incidence in the country areas was higher than that in the city tending to be more in the 7 to 10 year-old children and there were isolated cases in adults In total these city and country cases accounted for somewhat less than half of *the national total*

It is not easy to explain why this epidemic occurred in Cork Cork is a port having a busy passenger traffic with the United States and there is also a frequent boat service to Britain About 6 weeks before the epidemic an International Film Festival had been held in the city and Cork had been the center of several other national conferences There is a pure water supply to the city but crude sewage is discharged untreated at many points straight into the river which runs through the city center This river is highly polluted but the epidemic was unassociated with bathing All these factors with the exception of the Film Festival have been part of Cork life since at least the end of World War II One must presume the introduction of an invasive virus into a relatively nonimmune child population Virus isolation was not attempted for every case but all viruses isolated were of Type 1

Public health measures to control the spread of the infection were those advocated in the *First Report of the W H O Expert Committee on Poliomyelitis* and while it was felt that what was being done was logical the results did not seem to be impressive

During 1956 a survey of antibody levels against poliomyelitis was carried out by the Irish Medical Research Council Sera were collected from children on their first admission to the hospital and all the specimens had been taken before the epidemic began in the second half of the year In addition a number of adult sera were collected at blood transfusion donor clinics These specimens were representative of the different areas in the country and the *numbers in each age group were as follows*

0– 4 years	86
5– 9 years	159
10–16 years	125
18–30 years	100

The final report is not yet available but taking the country as a whole the results in the 5 to 9 year group show 43 per cent of children with antibodies to Type 1 51 per cent to Type 2 and 62 per cent to Type 3 The results for Type 3 are somewhat unexpected as only a small number of strains of this type have so far been isolated in Ireland As a result of the experience during the second half of 1956 the level of Type 1 antibody may now be some*what higher as nearly 80 per cent of the polio*myelitis viruses isolated during that period proved to be Type 1 and it is clear that this was the type responsible for the 1956 epidemic

At the beginning of 1956 our 3 main acute poliomyelitis treatment centers had been staffed and equipped Of the 499 paralytic cases the great majority were treated at one or other of these centers about 13 per cent requiring treatment for breathing or swallowing difficulties One of our major problems in the basing of our treatment on these 3 centers is the large area each center must serve It is difficult to obtain agreement on the best methods of transporting acutely ill cases to the centers We have constructed a large ambulance which carries an electrically operated tank respirator and has facilities for intermittent positive pressure and suction This ambulance is staffed by an anesthetist a physician and a nurse It is situated at Dublin the center serving by far the largest district The other 2 centers rely on their ordinary ambulances which are staffed with similar

teams and use one or other form of manually assisted respiration if required to bring their cases from their homes to the center

Twenty deaths attributable to poliomyelitis occurred during 1956 giving a rate of 4 deaths per 100 paralytic cases This compares with the rate of 1? per 100 paralytic cases in previous years before the regionalization of treatment It is very doubtful if all deaths due to polio are certified accordingly Some deaths shown on postmortem to have been caused by polio have baffled even the most astute clinicians and had been thought to be due to another cause

Since 194? we have had a growing pool of persons crippled by this disease The Minister for Health has set up the National Organization for Rehabilitation to advise him on the various problems involved So far this work is in its infancy

In May 1957 vaccination against poliomyelitis was introduced for the first time using a British manufactured vaccine For a number of reasons the vaccine has been offered to the 1 and 2 year-olds initially The course consists of 3 1 ml injections given intramuscularly the first 2 at about a 4 week interval and the third at least 7 months later This vaccination scheme was offered free of charge to those in the eligible groups and of 125 000 infants in these groups the parents of 11 000 have applied for vaccination We have now extended the scheme to children under 5 years

ISRAEL

Dr P Yekutiel

The transition of poliomyelitis from a sporadic to an epidemic disease began in Israel in 1950 with a formidable epidemic of 1 600 cases representing an attack rate of 146 per 100 000 Ever since the annual incidence has never been below 30 per 100 000 with an average of 4? for the last 5 years—one of the highest rates in the world

The age distribution of our cases of infantile paralysis is similar to that in other countries in the Middle East The highest age specific rates are found in the first and the second year of life and almost 80 per cent of all cases are in children under the age of 3 This age distribution has not changed since 1950

The hospitalization and the rehabilitation of poliomyelitis cases is a major burden for the country All cases are hospitalized free by the government a large rehabilitation center has been developed in one of our government hospitals

In view of this serious poliomyelitis problem the Ministry of Health began the planning of mass vaccination as soon as the encouraging results of the Salk vaccine trials in the U S A were published in April 1955 As it soon became clear that supplies of vaccine from abroad could not be relied on for an uncertain period these plans included the first steps for the local production of a Salk type vaccine in Israel Preliminary tissue-culture work was carried out in collaboration by several virus laboratories In January 1956 the Ministry of Health s special poliomyelitis vaccine laboratory was opened Actual production of vaccine did not start until April of the same year since only then after many delays and diffi ulties did we receive the first shipment of monkeys Yet in January of this year the first lot of trivalent formolized vaccine tested for safety and potency both in Israel and abroad was used in our vaccination campaign

Our first antipoliomyelitis vaccination program comprised infants and children from the age of 6 months to 3 years Two basic inoculations were given during the win er period January to April of this year with the booster injections scheduled for this autumn The response of the public to the voluntary program was beyond our expectations of the 123 000 children in the eligible age group 115 000 i e more than 90 per cent received their first 2 injections This is the more remarkable since we were not dealing with school children who are regimented much more easily for such vaccination programs

In order to save vaccine and relying on the experience in Denmark we gave intradermal injections of 0.3 cc to a large proportion of the children and 0.5 cc subcutaneously to the others In addition to the local vaccine an equal amount of Salk vaccine from the U S A released by the U S. Publ c Health Service was used in the program

It is of course too early yet for a definite evaluation of the results of this vaccination in Israel However the following data are of interest Our poliomyelitis season starts early

reaching its peak between May and July The average number of cases during the February to June period over the last 5 years was 270 the minimum number was 106 This year the total number of cases over the same period was 13 of which 8 had not been vaccinated The total number of poliomyelitis cases in vaccinated children aged 6 months to 3 years during these 5 months was 5 representing an attack rate of 0 43 The attack rate in nonvaccinated children of the same age group was 4 3

The Israel Government is grateful to virologists of the U S A and Denmark without whose valuable help and advice the local production of vaccine could not have succeeded in such a short time

ITALY

Prof Giuseppe Penso

La poliomyélite continue a etre en Italie une maladie de la premiere enfance La plupart des cas declares sont en effet des enfants ages de moins de 4 ans Le nombre de cas declares s eleve par annee a environs ? 700 sur une population de 50 000 000 environ d habitants Malgré le nombre limite de cas le virus polio est tres repandu chez les enfants sains D apres les recherches faites a l Institut Superieur de la Sante de Rome il resulte que dans les communautes de nombreux enfants sont porteurs de virus polio virulents Dans certaines communautes exemptes de poliomyelite on trouve jusqu à 75 / de porteurs de virus poliomyelitiques virulents

En Italie on a eu plusiers epidemies de meningo neuraxite dues a differents types de *Coxsackies* entre autre a un virus *Coxsackie A* dont le typage à ete impossible avec les 19 types d antiserum A D autres cas de meningoneuraxite on a isole des virus cytopathogenes dont le typage a ete impossible

Cette annee on a commence en Italie la vaccination contre la polio avec un vaccin etudie et prepare en Italie et controle par l Institut Superieur de la Sante La reponse immunitaire a ce vaccin a ete tres bonne

La vaccination contre la poliomyelite n est pas obligatoire en Italie et elle est pratiquée gratuitement par l Etat aux enfants qui vivent en communautes On vaccine seulement au dessous de 4 ans

JAPAN

Dr Masami Kitaoka

At the last Conference the outline of polio in Japan was announced and it was mentioned that a few Japanese investigators had started studies on the disease in laboratories expecting fruitful results in the future Since then information has been received concerning the isolation of the virus from various samples the identification of new isolates the immune state of inhabitants on sera collected from certain age groups in different areas all over Japan and also the studies on tissue culture carried out on the basis of cytopathogenic effect of the polio virus on the growing cells at several laboratories for example in Sapporo (Dr Kanamitsu) in Tokyo (Drs Matumoto Takatsu Kono and Nagaki) in Matsumoto (Dr Yamada) in Osaka (Drs Nishizawa and Tatsumi) and in Kumamoto (Dr Rokutanda) Our institute has been functioning as the WHO Poliomyelitis Regional Center in the northwest Pacific area since September 1955 not only for the epidemiologic survey in the field but also for the laboratory work

As for the incidence of polio in Japan a few thousand cases per year paralytic and non paralytic with a fatal case rate of approximately 20 per cent were reported according to the Welfare Ministry since 1948 when the disease was classified as being notifiable in Japan The number of reported cases reached the maximum of 4,233 (4 7 rate per 100 000 inhabitants) in 1951 and then decreased gradually in number year by year following neither vaccination nor special preventive procedures In 1955 and 1956 the number of cases reported was 1,314 and 1 497 respectively (roughly one third of those in 1951) although 2 epidemics one in Sapporo Hakkaido Prefecture and the other in Shuso Ehime Prefecture occurred in the last year All new isolations from the cases in both epidemics were identified as Type 1 polio viruses Japan is still strongly endemic of polio virus as already pointed out by Dr Paul about 10 years ago because almost all cases were in children under 4 years old The morbidity rate in Shuso (0 69 /) was twice as high as that in Sapporo among the age group under 4 years and it is surprising that 1 year-old children suffered from polio paralytic and nonparalytic by

the ratio of 1 108 per cent in Shuso The ratio of paralytic to nonparalytic polio cases was 3 1 in Sapporo and vice versa in Shu o This might be due to different sampling in both areas that is cases in Sapporo were reported by local phy sicians while those in Shuso were found by checking personally each inhabitant by a case finding team

In the laboratory virus isolation has been undertaken continuously from the patients in Aomori Tokyo and Osaka since 1954 As a result out of 134 samples (stools) 67 (50) polio viruses were found these consisted of 36 Type 1 (53 7 /) 20 Type 2 (29 8) and 11 Type 3 (16 4 /) Four nonpolio viruses (3) were isolated However the Type 2 virus was encountered more often in the stools from the patients (not in epidemic cases) in Aomori and Tokyo especially in Osaka in 1957 contrary to results in Osaka in 1954 and 1956 It is inter esting to note that in Shuso Type 1 viruses were isolated by 40 per cent (22/55) from patients by 5 9 per cent (30/513) from all inhabitants of any age in epidemic areas and by 9 7 per cent (4/41) from children under 7 years old living in nonepidemic areas near Shuso When compared with positive isolation rates in each age group among children under 7 years old of three groups (patients children in epidemic areas and children in nonepidemic areas) the children under 5 years old gave little less posi tive rate (about 30) in epidemic area and much less (about 10) in nonepidemic areas than the patients (about 45) while in the age groups of more than 7 years old such dif ferences among 3 positive rates were not so marked

On the other hand virus isolation was at tempted from the stools collected from healthy children in Aomori Yamagata and Tokyo Six Type 2 polio viruses were isolated from 55 infants under 1 year old at the Setagaya Infant Home in Tokyo The youngest virus harboring infant was 2 months and 29 days old

During the virus isolation experiments viruses giving a cytopathogenic effect on the cul ture cells but not neutralized by the immune serum of any type of polio virus were encoun tered The cytopathogenic effect of such new isolations on human embryo cells HeLa cells monkey kidney cells and human amnion cells as well as the results of identification are tabu lated though those experiments are not yet all completed Out of 16 strains 5 were identified as adenoviruses (Type 1 1 Type 2 3 Type 5 1) 1 as a Coxsackie virus and 6 as ECHO viruses (both latter viruses not yet typed) The remaining 4 are still unknown

The immune state of inhabitants was sur veyed against polio virus (Drs Takatsu Ya mada and Kono) and against Coxsackie virus (Drs Fukumi and Hamagami) Furthermore the usefulness of complement fixation test on polio the practical use of color change (pH change) of culture media and the plaque form ing on monolayer of cells after inoculation of polio virus the mutant polio virus resistant to the inhibitor in bovine serum HeLa cell adapted ECHO virus the plaque forming of ECHO virus on monolayer of HeLa cells and ECHO virus resistant HeLa cells were investigated

As for vaccine a killed vaccine (like Salk s vaccine) has not yet been prepared in Japan However the immune response both comple ment fixing and neutralizing antibodies was checked in Japanese infants from 6 months to 4 years old (mostly 1 to 2 years old) following 3 shots of a 1 ml subcutaneous dose or an 0 1 ml intradermal dose the first 2 shots given 7 days apart and the third shot as a booster given 6 months later using the vaccine made in the U S A The placebo vaccine was prepared in my laboratory from tissue culture without virus inoculation The results so far obtained were almost similar to those reported by Dr Francis that is the poor response against Type 3 virus The avirulent strain of Type 2 polio virus was reported at the last conference but such strains had not proved to be avirulent after intraspinal inoculation After all any completely avirulent strain has not yet been obtained in Japan

KENYA

Dr J R Harries

The first large epidemic occurred in Kenya in 1953 1954 Approximately 700 paralytic cases were notified during that year The incidence among the various racial groups is interesting

Europeans	256 per 100 000
Asians	60 per 100 000
Africans	12 per 100 000

Since December 1956 we have been experiencing a further epidemic So far 400 paralytic cases have been notified During the past 3 weeks 60 cases have been notified In Nairobi about 30 cases a month have been admitted since the beginning of the year

The majority of cases occur in the towns starting at the coast in Mombasa spreading to Nairobi then proceeding up country

Only Type 1 virus has been isolated from any of our paralytic cases

In 1954 a respiratory unit was set up in Nairobi This unit accepts cases from all over Kenya Uganda and Tanganyika As the roads in Kenya are bad and large distances have to be covered an air rescue service has been set up to transport respiratory cases Approximately 20 flights have been made

During this year we have admitted 45 cases of respiratory paralysis there have been 7 deaths Ten per cent of these cases had bulbar paralysis 20 per cent had bulbospinal paralysis and 70 per cent had diaphragmatic or intercostal paralysis

Vaccination against poliomyelitis was started this year using the Salk vaccine So far 4 000 children have been vaccinated but we are experiencing difficulty in obtaining adequate supplies of vaccine

LEBANON

Dr Joseph Hatem

Avant 1939 la poliomyelite etait tres rare au Liban presqu inexistante C est à partir de cette date que l attention des responsables fut attiree par l apparition de quelques cas localises dans la region du sud du pays aux confins de la Palestine Jusqu en 1951 l indice de morbidite pour 100 000 habitants ne dépassait pas 0 40

En 1952 le nombre de cas declares passe brusquement a 58 soit un indice de morbidite de 4 6 et apres des variations atteint en 1956 le chiffre record de 82 cas soit un indice de 6 5 Nous ne savons pas exactement la raison de ce changement brusque et de cette extension de la maladie il coincide toutefois avec l arrivee dans la capitale d un grand nombre de ressortissants etrangers d ailleurs les chiffres cites plus haut comprennent les cas survenus chez ces etrangers en residen e temporaire dans le pays

Il est a remarquer que les chiffres cites plus haut sont inferieurs aux chiffres reels car l ordre de declaration de la poliomyelite n est pas toujours respecte De plus tous les diagnostics concernant les cas rapportes sont des diagnostics cliniques

Quant a la frequence saisonniere on peut constater qu il existe une morbidite maxima en 1953 au printemps et en ete en 1954 en ete et en automne en 1955 au printemps jusqu a l hiver en 1956 principalement en eté

En tenant compte des realites climatiques nous pouvons donc deduire que la poliomyelite au Liban a une predilection pour la periode chaude et seche periode de 6 mois environ qui va de mai a octobre avec un maximum en juin juillet et aout, c est a-dire aux mois qui correspondent a l ete classique

Nous regrettons de ne pas disposer de chiffres statistiques valables sur la mortalite la repartition suivant l age et la gravite des paralysies

En outre il existe des cas sporadiques durant les mois froids et humides de novembre a avril

Il resulte que depuis 1952 l augmentation relativement considerable de la morbidite de la poliomyelite pose un probleme nouveau au Liban

Dans la lutte antipoliomyelitique que nous devons mener desormais quels sont les moyens dont nous disposons?

Du point de vue hospitalier il faut reconnaitre que nous manquons de facilites et de personnel specialise pour soigner les maladies graves a la phase aigue Nous ne disposons que de quelques poumons d acier et si par malheur une grave epidemie se produisait nous serions a court d appareils et de personnel specialise

Par contre en ce qui concerne les sequelles et la readaptation fonctionnelle un centre de reeducation fonctionne deja a Beyrouth Il a ete cree grace au concours de l Abbe A Cortbaoui de la Cite des Apprentis l Union libanaise de la Protection de l Enfance les deux facultes de Medecine de Beyrouth le Ministere de la Sante l U N I C E F et l O M S Ce centre est un modele du genre Il est bien equipe et dote d un personnel qualifie D autres centres de ce genre sont en voie d achevement En outre trois hopitaux prives de Beyrouth ont dejà leur departement pour la réadaptation des infirmes-moteurs

Enfin la mise en marche imminente du Laboratoire National d Hygiene Publique nous per

mettra dans un proche avenir de pouvoir isoler des souches et de déterminer les types predominants responsables de l'infection et surtout de mener une enquete serologique afin de determiner la protection de la population suivant les ages et decider de l'eventuelle necessite d'une vaccination antipoliomyelitique appliquée aux groupes d'age les plus exposés

NETHERLANDS

Dr H J Dykhuis

In 1956 more than 2,200 cases of poliomyelitis were registered in the Netherlands This number was the largest ever to wit 2,206 This means an incidence of 20 4 per 100 000 inhabitants

The year 1943 follows immediately with 1,931 cases but the morbidity per 100 000 inhabitants was higher to wit 21 3

In 1956 epidemic outbreaks of virus meningitis (caused by the ECHO virus) were also reported all over the country This certainly interfered with the diagnosis of nonparalytic poliomyelitis

Nevertheless in 1956 proportionally no larger number of nonparalytic cases has been notified A 4 times larger number of paralytic cases is usual in the Netherlands Last year this was also the case

With regard to the distribution according to sex it can be mentioned that a larger number of cases was reported among men than among women

As to age the largest number of paralytic cases was recorded among the youngest age groups to wit 60 7 per cent from 0 to 4 years of age and 24 8 per cent in the group from 5 to 9 years of age This age pattern is rather constant in our country A shift to a higher age group did not occur

The first symptoms of an epidemic occurred in the 21st week and the peak was reached in the period from the middle of July to the middle of August A year with a much higher morbidity than average preceded 1956 a fact that was also noted in earlier epidemics. This occurrence made it possible for us early in 1956 to be alerted to the possibility of an outbreak of an epidemic so that measures were taken in order to be able to fight its consequences The predominant idea was that modern treatment of poliomyelitis required that care should be given in special centers where the best care is available because teamwork is possible and modern apparatuses are used This applies especially to patients with respiratory distress

With the aid of the Government 9 polio centers were set up which provide mechanically functioning respirators from a pool In this way interchange or completion of respirators was possible In these centers a total of 230 patients with respiratory paralysis were treated i e about 10 per cent of the total number of cases registered The mortality of the patients treated with respirators was no more than 20 per cent (Up to now 50 patients are being treated totally or partially with respirators 18 of whom probably will be in permanent respiratory distress)

Besides these centers a number of rehabilitation clinics were rapidly established where individual physical-therapeutic treatment in small units was especially accentuated At the same time possibilities for ambulatory treatment were created

In 1956 the central authorities the sickfunds and the national private organization Het Prinses Beatrix Poliofonds gave financial support in order to fight poliomyelitis On a voluntary basis the population collected large sums of money on behalf of the fund

With regard to vaccination against poliomyelitis it can be mentioned that in the autumn of this year a vaccination program will be started which will be directed and organized by the central authorities At first this program will be limited to one age group Next spring the number of children to be vaccinated will be increased Vaccination will not be compulsory In the meantime some municipalities and industries and also a certain number of private practitioners have started vaccination at their own initiative The Government buys vaccine in the U S A Also Belgian vaccine is being imported In the State Laboratory and in collaboration with the Netherlands Institute for Preventive Medicine a pilot study is being made with regard to the production of a Dutch vaccine

Up to this moment only a limited number of cases of poliomyelitis have occurred in 1957

NORWAY

Dr Peter M Holst

Since the last Conference in 1954 Norway has had comparatively few cases of polio In 1954 the number of paralytic cases was 434 in 1955 191 and in 1956 only 73 (Table 16) Until now we have had only a couple of cases this year

Vaccination was started November 1 1956 using American and Danish vaccines The vaccination was offered to all school children in their first 7 years on a purely voluntary basis Up to this time it is officially reckoned that about 90 per cent of 450 000 children belonging to these age groups have been completely vaccinated After January 1 any person may be vaccinated by his private physician and we now reckon that an additional 400 000 persons are partially vaccinated In many cases municipal authorities or industrial concerns have paid for the vaccination From September 1 the State will offer free vaccination to all children in the age group of 9 months to 2 years The Danish vaccine has been administered intracutaneously in the way described by my colleague Dr Juel Henningsen The American vaccine has been applied subcutaneously From different parts of the country about 5 000 blood samples have been collected in order to test the antigenic response of the vaccines *These samples have* been taken before vaccination and after the second and the third vaccination Until now only very few of them have been examined *but results show a good response It is too* early to predict the effect of the vaccination on the epidemiology of the disease Complications have not been registered

The facilities for virus and serologic research have been very limited in Norway but conditions in this respect will improve considerably in the course of this and next year So far we have no plans for producing vaccines

Thanks mainly to our National Foundation against Poliomyelitis conditions of treatment and aftercare have been much improved during the last years We have now 10 clinics for the treatment of poliomyelitis *This year the Foundation* has opened a central clinic in Oslo which will have facilities for orthopedic surgery and neurologic and physical therapy The total number of beds which are reserved exclusively

Table 16 Number of Cases of Poliomyelitis *with or without Paralysis in Norway* from 1950 to 1957

Year	Paralytic Polio	Nonparalytic Polio
1950	706	199
1951	1 563	670
1952	527	197
1953	928	179
1954	434	150
1955	191	58
1956	73	24
1957	6	1

for the treatment of polio cases is now 300 Qualitatively but not quantitatively we have a very difficult problem which I suppose we share with other nations What shall we do with our young respiratory cases? We have 25 to 30 young patients who are more or less dependent on artificial respiration We feel that it would be highly unsatisfactory to bring these patients home even if it is technically possible because in their homes they will be completely isolated and they will have practically no chance to develop their minds These patients will never be able to support themselves by manual work but most of them are intelligent We feel that we must give them something to live for But how? Any suggestion will be received with thanks

PHILIPPINE ISLANDS

Dr Benjamin V Tamesis

The consensus in the Philippines today by workers in public health is that poliomyelitis is not a public health problem This opinion has probably been distilled from the following observations

1 The absence of epidemics in the Philippines that would approximate the extensiveness of poliomyelitis epidemics in temperate zones

2 The belief that the virus of poliomyelitis is endemic in the Philippines

3 The further assumption that the population as a whole has acquired a certain degree *of immunity because of its continued contact* with poliomyelitis virus

While this attitude would tend to mitigate a inst any active public health measure against elitis, there are certain basic facts which

TABLE 17 POLIOMYELITIS CASES AND DEATHS PER 100 000 POPULATION IN MANILA 1939 1955

Year	Cases		Deaths	
	No	Rate	No	Rate
1939	26	4 20	—	—
1940	63	9 56	5	0 76
1941	3	0 43	1	0 14
1942	16	2 17	4	0 54
1943	21	2 70	3	0 39
1944	4	0 49	2	0 24
1945	8	0 93	3	0 35
1946	5	0 59	3	0 33
1947	16	1 71	9	0 96
1948	14	1 44	7	0 72
1949	5	0 49	3	0 30
1950	176	11 97	16	1 52
1951	29	2 65	5	0 46
1952	59	5 21	9	0 80
1953	42	3 59	9	0 77
1954	156	14 89	28	2 31
1955	102	8 16	16	1 28
Total	695	—	123	

TABLE 18 AGE INCIDENCE OF POLIOMYELITIS 1950-1954 (CASES FROM SAN LAZARO HOSPITAL)

	No of Cases	Percentage
Below 1 year	179	19 48
1 to 4	564	61 37
5 to 9	50	5 44
10 to 14	35	3 90
15 to 19	28	3 04
20 and above	63	6 86
Total	919	100

present themselves as far as poliomyelitis is concerned in the Philippines

1 There is no accuracy as is true in some areas in the reporting of poliomyelitis cases with the consequence that any available data during the time of sporadic outbreaks are data available only as far as cases that come to government hospitals are concerned It is safe to assume that for every case that comes to a government hospital for treatment there are many other cases unreported The conclusion is unavoidable that whatever statistical data the country has today is in all probability inaccurate and does not present a true picture of the disease (Table 17)

2 The available statistical data would seem to indicate that the ravages of polio hurt the age group between 1 to 5 years the most and that the incidence of the disease diminishes with the age of the individual (Table 18)

3 From the available records of the National Orthopedic Hospital it would seem to appear that a good amount of the orthopedic work involves giving attention to postpolio patients with paralysis

4 There are hardly any technical personnel with extensive and sufficient training in the task of rehabilitating the polio paralytic in our country

Therefore we are forced to the conclusion that the consensus as obtains in my country today may be wrong in the sense that

1 There are more victims with paralysis than are reported in official statistics

2 If the age group that bears the brunt of the disease is the one composed of the children from 1 to 5 years of age this very same reason would indicate that preventive measures should be undertaken by our government to prevent or at least diminish the incidence of crippling that this disease leaves in the 1 to 5 year-old age group

3 The third problem that comes up is what to do for those who have already developed the paralysis and the deformities from polio The National Orthopedic Hospital is almost alone in its attempt at providing physical medicine modalities for the management of postpolio paralysis and in its pioneering in the reconstructive procedures that are usually available to the victims of polio paralysis in more advanced countries This effort however is in its infancy and will require considerable attention so that the lessons that our orthopedic surgeons have learned may be channeled correctly and or implemented by the developments in this type of work in the more experienced countries

There is at present a civic organization in our country the Philippine Foundation for Polio headed by General Basilio J Valdes which has attempted to carry on polio work However its efforts have been restricted because its funds are limited and it has concentrated its work mostly on direct material aid in the form of hospital materials equipment and br becomes evident that we have only t

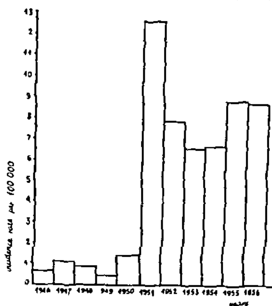


Fig 26 Poliomyelitis incidence rate per 100 000 in Poland 1946-1956

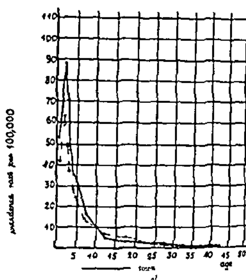


Fig 27 Distribution of poliomyelitis in Poland by age in rural and urban areas 1951 1953

surface of the entire problem but we are hopeful that in the increasing consciousness of our people for the physically disabled the civic organization in our country devoted to polio eventually will become stronger and more active in its function

And this is the thought I should like to leave While my government is faced with the more acute problems of tuberculosis yaws malaria and schistosomiasis and while the attention of poliomyelitis workers is presently occupied for the most part by virus studies and efforts at providing a more effective prophylaxis our main problem in the Philippines today is the provision of sufficiently trained personnel for distribution to the rest of the country so that the deformities left behind by our small sporadic epidemics—old and recent—can be attended effectively—in other words the total physical rehabilitation of the paralytic polio patient

In closing on behalf of the Philippine Government I wish to thank the Swiss Government and the International Society for Poliomyelitis for the opportunity to be present at this conference

POLAND

Prof Dr F Przesmycki

The incidence of polio in Poland during the years 1926 to 1951 was rather low and did not exceed the figure of 1.5 per 100 000 But in 1951 an epidemic broke out which reached the rate of 12 7 per 100 000 During the next years the variation in the rate was rather insignificant, and the number of cases ranged from 6 6 to 8.9 per 100 000 (Fig 26)

In the epidemic year the number of cases was 3 184 and the corresponding figure for 1956 was 2 418 The average lethality for the years 1951 to 1954 was 6.3 per cent. The number of cases in males was higher than in females From 1951 to 1956 the cases in males rose to 55.2 per cent and those in females to 44 8 per cent of patients

The analysis of cases by age groups shows that the highest incidence rate was seen in the age group 0 to 9 years (about 90 % of all notified cases) The distribution of the cases in percentage by age groups in 1956 was as follows

0 to 4	78 6%
5 to 9	12.8%
10 to 14	2 8%
15 to 19	2 1%
Over 20 years	3 6%
Age not stated	0 1%

The percentage distribution of cases in 1956 is similar to that registered during the years 1951 to 1955

The incidence of polio is higher in urban than in rural districts (Fig 27)

To the cases notified in 1951 to 1956 the following clinical classifications could be applied

Paralytic cases	90 5%
Nonparalytic cases	6 7%
Abortive	2 8%

The percentage of paralytic cases is higher in Poland than in other countries However it should be noted that nonparalytic cases or cases with slight manifestations of paralysis often are not reported and this is probably responsible for the divergency between our data and those of foreign countries

The investigations made in 1951 show that the epidemic was due to a Type 1 virus Four strains were isolated from feces on monkeys They belonged to Type 1 In 1956 there were isolated 131 strains on tissue cultures The investigations were carried out in Warsaw and in some other towns The distribution of isolated strains by types is given below

Type 1	82	66 6%
Type 2	31	25 2%
Type 3	10	8 0%
Orphan strains	8	

Recently the production of vaccine by the Salk method was inaugurated The first series of this vaccine have already been prepared. It is intended to proceed to an experimental vaccination of 100 000 persons during the current year

RUMANIA

Dr C Ionesco-Mihaiesti

Dans l'evolution de la poliomyelite en Roumanie on peut considerer 2 periodes distinctes au point de vue épidemiologique une premiere, de 1927 a 1949 et une deuxieme période de 1949 à 1957

La premiere periode commence avec l'epidemie de 1927 1928 première grande epidemie enregistree dans notre pays (plus de 2 200 cas paralytiques)—une morbidité de 11,3 cas pour 100 000 habitants

Pendant les 21 annees non-épidémiques que suivirent depuis 1928 à 1949 les statistiques officielles indiquent une morbidité qui n a jamais depassè 2 6 pour 100 000 et oscillant en general entre 0,2 à 1 5 pour 100 000 habitants

La derniere periode de 1949 à 1957 (1 janvier) est caracterisee par un rapprochement impressionnant des annees epidemiques—d une

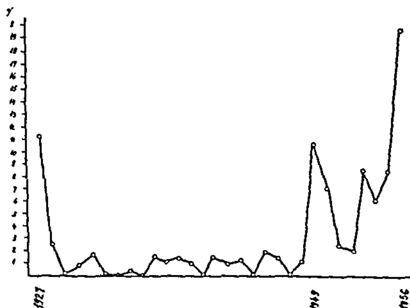


Fig. 28 Morbidite de la poliomyélite depuis 1927 en Roumanie (taux par 100 000 individus)

part et d autre part par l installation d un etat endemique avec une incidence generale de la poliomyelite relativement eleve sur lequel appa raissent des crochets epidémiques assez impor tants

Apres 1949 annee quand survint le deuxieme grande epidemie de poliomyelite avec une morbidite de 10 5 pour 100 000 habitants les annees epidemiques se succedent de pres 1950 avec 7 2 pour 100 000 1953 avec 8 pour 100 000 1955 avec 8 4 pour 100 000 habitants situation qui en 1956 atteint l incidence considerable de 18 7 pour 100 000 habitants (plus de 3 200 cas confirmes)—ce qui represente la morbidite par poliomyelite la plus elevee consignee jusqu à present en Roumanie (Graphique 28)

L extension de la poliomyelite dans notre pays et cette incidence si elevee de la morbidite pendant la periode de 1949 à 1957 s est produite à la faveur de foyers epidémiques delimites qui n ont jamais interésse plus d approximativement un tiers de la surface totale du territoire du pays

Malgre leur etendue relativement reduite ils presentaient toutefois une incidence de la mor bidite tres elevee

C est dans ces conditions d endemo epidemi cite qu a éclate l epidemie de 1956 qui marque probablement une nouvelle etape dans l evolu tion de la poliomyelite dans notre pays

Consideree dans son ensemble l epidemie de 1956 tout en gardant certains aspects epidemio logiques de la periode 1949 1956 presente un envahissement massif de certaines regions rela tivement bien delimitees dont 2 dans la zone centrale du pays 1 dans le nord-ouest 1 autre dans le sud-ouest et une cinquieme dans le nord est.

Le total des cas enregistres dans ces 5 regions represente 60 % du nombre total des cas apparus en 1956 tandis que la population de ces regions ne represente que 27 / de toute la population du pays La morbidite dans ces regions a varie entre 32 à 54 pour 100 000 habitants et dans certains districts a depasse 100 pour 100 000 habitants Il faut ajouter que ces regions sont tres industrialisees pourvues d un important reseau de voies de communications et caracte risees par une circulation tres active de la population

L analyse de la morbidite genérale étudiee separement pour les villes (21 2 %) et les vil lages (17 4 %) nous montre que l incidence dans le milieu rural est tres élevee du nombre total des cas de poliomyelite 35 / proviennent des villes et 65 du milieu rural L extension dans le milieu rural pendant l epidemie de 1956 a dans la plupart des cas pris un caractere explo sif comparable a celui des epidemies de rougeole ou aux infections d origine hydrique

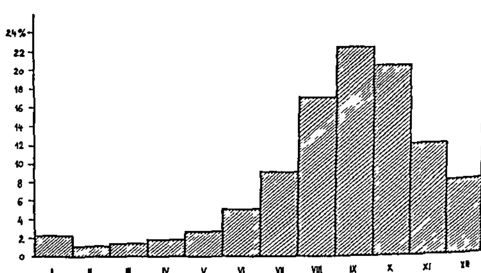


Fig 29 Repartition saisonniere des cas de poliomyelite en 1956 (pourcentage par mois de nombre total de cas)

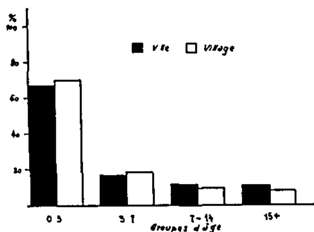


Fig 30 Repartition par groupes d age des cas de poliomyelite declares dans les villes et dans les villages

En ce qui concerne le caractere saisonnier on constate comparativement aux annees epidémiques antérieures un deplacement de l incidence maxima dans les mois de septembre et octobre (Graphiques 29 30 et 31)

Un autre caractère que nous voudrions signaler est la frequence des cas de poliomyelite parmi les enfants d audessous de 6 mois (96 cas) 59 parmi ces enfants presentaient des formes paralytiques spinales 6 paralysies des nerfs craniens 11 formes respiratoires et 70 seulement des formes nonparalytiques

Nous n insisterons pas sur les formes cliniques et leur repartition par groupes d age

Le nombre de déces en general pour 100 malades a ete 7 4

Répartis par Groupe d Age	Total Decès	Décès pour 100 Cas par Groupe d Age
0 3	69%	7 7
3 7	15%	6 8
7 14	8%	6 0
15 et plus	8%	9 1



Fig 31 Repartition saisonniere des cas de poliomyélite par âge (pour centage par groupes d age du nombre as par mois)

La proportion de deces pour 100 malades montre la gravite de l epidemie de 1956 La frequence elevee de ces deces en milieu urbain comparativement au milieu rural tient en grande partie au nombre plus élevé des malades de plus de 15 ans en milieu urbain

Avant de terminer ce court expose je voudrais mentionner quelques resultats de nos examins, effectues à l Institut Dr J Cantacuzene sur plus de 550 feces provenant de malades ou de leur entourage pour la mise en evidence du virus et la determination des types

Nous avons isole 114 ouches dont

110 Type 1	(96 49%)
1 Type 2	(0 87%)
3 Type 3	(2 64%)

Nous n avons jamais rencontre la coexistence avec les virus Coxsackie

Je me permets en terminant ce rapport de feliciter et remercier au nom de notre Gouvernement l Association Nationale pour la Poliomyelite des Etats Unis d Amerique pour l organisation de cette quatrieme Conference et d exprimer la gratitude de notre pays pour l hospitalite qui nous a ete offerte par la Suisse

SWEDEN

Dr Gunnar Olin

A severe outbreak of polio occurred in Sweden in 1953 with 4 7 paralytic cases per 100 000 during the epidemic year from April 1953 through March 1954 Since then the polio incidence has been low and slowly decreasing In 1954 there were only 5 cases per 100 000 in 1955 4 cases in 1956 3.5 cases per 100 000

The big outbreak of 1953 offered extensive opportunities for clinical studies of the disease and for investigations concerning its treatment The Stockholm hospital for epidemic diseases has made an important contribution in this field

Much interest has also been devoted to the problem of vaccination against polio by a team in Stockholm A Swedish polio vaccine produced from virus cultivated on human embryonic tissue was tried on 2 000 children early in 1955 No untoward reactions were observed However the Cutter incident as well as findings in Swedish control tests necessitated improvement of the safety of the vaccine It has also been considered important to try to increas the immunizing capacity of the vaccine Owin to the work connected with the solution o these problems large scale production has beer retarded

Therefore the first immunization progran had to be carried out mainly with vaccine fror the U S A released by courtesy of the federa authorities When the immunizations started ir February of this year only about 100 liters o Swedish vaccine produced from virus cultivated on monkey tissue were available The program included children aged 4 to 11 years About 900 000 children were offered vaccination free of charge about 700 000 accepted and by now have received 2 inoculations each of 1 ml 3 weeks apart The program has been accomplished without an accident

The immunizing capacity of the vaccines used has been titrated both in children and in guinea pigs The result is presented at the Scientific Exhibit of the Conference

Since more Swedish vaccine became available in May it has been possible to extend the vaccination to certain categories at risk namely the staffs of virus laboratories and hospitals for epidemic diseases pregnant women and on a smaller scale people aged below 35, at their own expense The conscripts on duty in the armed forces have also been vaccinated

By extensive serologic investigations it has been shown that the sero immunity against poliomyelitis is developed rather slowly in the younger age groups in Sweden seronegative individuals are found even in the higher ages Therefore vaccination is needed up to the age of 40

In Sweden much work has also been devoted to the study of enteric Coxsackie and ECHO viruses Especially their connection with outbreaks of aseptic meningitis has been investigated

SWITZERLAND

Prof Dr O Gsell

Fur die Schweiz ist die epidemische Poliomyelitis heute die wegen ihrer Dauerschaden gefahrlichste jahrlich wieder auftretende Infektionskrankheit Die von der Schweizerischen Vereinigung gegen die Poliomyelitis und vom Eidg Gesundheitsamt gemachten Erhebungen ergeben folgende Resultate

MORBIDITÄT

Die Poliomyelitis in der Schweiz ist seit 1930 in stetem Zunehmen begriffen. Die Zahl der gemeldeten Fälle betrug in den Jahren 1926-1930 im Durchschnitt 3,7 Fälle pro 100 000 Einwohner. In den Jahren 1951-1955 erreichte der Durchschnittswert bereits 19,6 Fälle pro 100 000 Einwohner (absolute Zahlen: 1954 1678, 1955 919, 1956 973).

MORTALITÄT

Die Sterbefälle an Poliomyelitis haben in den letzten 30 Jahren ebenfalls deutlich zugenommen, jedoch nicht im gleichen Umfang wie die gemeldeten Fälle. Dieser Umstand ist auf die Tatsache zurückzuführen, dass im letzten Jahrzehnt vermehrt aparalytischen Fällen gemeldet wurden. Die durchschnittliche Mortalität an Poliomyelitis in den Jahren 1926-1930 betrug 0,16, in den Jahren 1951-1955 jedoch bereits 1,66 pro 100 000 Einwohner (absolute Zahlen: 1954 114, 1955 60, 1956 59).

LETALITÄT

Die Letalität, d.h. das Sterberisiko der an Poliomyelitis erkrankten Personen hat sich von 21,8 in den Jahren vor 1930 auf [illegible] in den letzten 3 Jahren vermindert. Dieser scheinbare Rückgang der Letalität ist fast ausschliesslich durch das bessere Meldewesen und durch das Melden von aparalytischen Fällen bedingt.

ALTERSVERTEILUNG

Die Altersverteilung der Krankheits- und Sterbefälle für die Jahre 1951-1955 war die folgende:

von den an Poliomyelitis erkrankten Personen waren

55,6 Kinder unter 10 Jahren
23,[illegible] Jugendliche von 10-19 Jahre
20,[illegible] Erwachsene über 20 Jahre

von den an Poliomyelitis gestorbenen Personen waren

40,0 weniger als 10 Jahre alt
30,4 zwischen 10-19 Jahre und
29,6 20 Jahre alt und mehr

GEOGRAPHISCHE VERTEILUNG

Die Verteilung der Poliomyelitisfälle innerhalb der Schweiz war sehr ungleich. Auffällig ist eine hohe Morbidität in der Ostschweiz und im Mittelland und die relativ geringe Morbidität in der Westschweiz, im Alpengebiet und in der Südschweiz. Es wurde festgestellt, dass im allgemeinen in den Städten weniger Krankheitsfälle auftreten als in den umliegenden ländlichen Bezirken. Die grössten Morbiditätsunterschiede bestehen zwischen dünn und dicht besiedelten Landkantonen. In den letzteren überschritt der Durchschnittswert der letzten 5 Jahre 25 Fälle 100 000 Einwohner.

VIRUSISOLIERUNGEN

Virusisolierungen in Genf (Dr. Bonifas), in Bern (Dr. Krech), in Zürich (Dr. Lindemann) zeigen eine deutliche Prädominanz des Typus I des Poliomyelitisvirus. Bei den an Poliomyelitiserkrankten und Kontaktpersonen findet man am meisten Typ-I-Virus im Stuhl, nur in einem kleinen Prozentsatz Typ 2 und Typ 3. Unter 279 isolierten Poliomyelitisstämmen aus dem Stuhl durch Krech (1955 und 1956) fand sich Typ 1 in 90,3, Typ 2 in 5,0, Typ 3 in 4,7).

ANTIKÖRPER GEGEN POLIOMYELITIS

Zur Beurteilung der Seuchenlage sind serologische Untersuchungen auf Abwehrkörper unerlässlich. Es wurden in der Schweiz an verschiedenen Orten derartige Untersuchungen in grossem Ausmasse durchgeführt. Die Ergebnisse, welche in der wissenschaftlichen Ausstellung tabellarisch dargestellt sind, zeigen, dass in Gemeinden, die seit 1950 frei von Poliomyelitisfällen geblieben sind, ein grosser Prozentsatz der Kinder keine Antikörper gegen Typ I Poliovirus aufweisen. In Gemeinden, die viel Fälle seit 1950 zu verzeichnen haben, erreicht die Durchseuchung der Jugendlichen von 5-[illegible] Jahren bereits Werte von über 60, jedoch nur gegen Typ I. Ganz allgemein haben bis zum [illegible]. Altersjahr nur wenige Prozent der Kinder Antikörper gegen alle 3 Typen. Die serologischen Untersuchungen unterstützen die Annahme, dass Typ I als hauptsächlicher Erreger paralytischer Erkrankungen in Frage kommt, während die Typen 2 und 3 in der Bevölkerung fast ebenso häufig serologisch nachgewiesen, aber bei Erkrankten weit seltener als Typ I gefunden werden.

SCHUTZIMPFUNG

Da durchschnittlich weniger als 50 der Kinder unter 10 Jahren und weniger als [illegible]

La proportion de deces pour 100 malades montre la gravite de l epidemie de 1956 La frequence elevee de ces deces en milieu urbain comparativement au milieu rural tient en grande partie au nombre plus eleve des malades de plus de 15 ans en milieu urbain

Avant de terminer ce court expose je voudrais mentionner quelques resultats de nos examins, effectues à l Institut Dr J Cantacuzene sur plus de 550 feces provenant de malades ou de leur entourage pour la mise en evidence du virus et la determination des types

Nous avons isole 114 ouches dont

110 Type 1	(96 49%)
1 Type 2	(0 87%)
3 Type 3	(2 64%)

Nous n avons jamais rencontre la coexistence avec les virus Coxsackie

Je me permets en terminant ce rapport de feliciter et remercier au nom de notre Gouvernement l Association Nationale pour la Poliomyelite des Etats Unis d Amerique pour l organisation de cette quatrieme Conference et d exprimer la gratitude de notre pays pour l hospitalite qui nous a ete offerte par la Suisse

SWEDEN

Dr Gunnar Olin

A severe outbreak of polio occurred in Sweden in 1953 with 4 7 paralytic cases per 100 000 during the epidemic year from April 1953 through March 1954 Since then the polio incidence has been low and slowly decreasing In 1954 there were only 5 cases per 100 000 in 1955 4 cases in 1956 3.5 cases per 100 000

The big outbreak of 1953 offered extensive opportunities for clinical studies of the disease and for investigations concerning its treatment The Stockholm hospital for epidemic diseases has made an important contribution in this field

Much interest has also been devoted to the problem of vaccination against polio by a team in Stockholm A Swedish polio vaccine produced from virus cultivated on human embryonic tissue was tried on 2 000 children early in 1955 No untoward reactions were observed However the Cutter incident as well as findings in Swedish control tests necessitated improvement of the safety of the vaccine It has also been considered important to try to increase the immunizing capacity of the vaccine Owing to the work connected with the solution of these problems large scale production has been retarded

Therefore the first immunization program had to be carried out mainly with vaccine from the U S A released by courtesy of the federal authorities When the immunizations started in February of this year only about 100 liters of Swedish vaccine produced from virus cultivated on monkey tissue were available The program included children aged 4 to 11 years About 900 000 children were offered vaccination free of charge about 700 000 accepted and by now have received 2 inoculations each of 1 ml 3 weeks apart The program has been accomplished without an accident

The immunizing capacity of the vaccines used has been titrated both in children and in guinea pigs The result is presented at the Scientific Exhibit of the Conference

Since more Swedish vaccine became available in May it has been possible to extend the vaccination to certain categories at risk namely the staffs of virus laboratories and hospitals for epidemic diseases pregnant women and on a smaller scale people aged below 35, at their own expense The conscripts on duty in the armed forces have also been vaccinated

By extensive serologic investigations it has been shown that the sero immunity against poliomyelitis is developed rather slowly in the younger age groups in Sweden seronegative individuals are found even in the higher ages Therefore vaccination is needed up to the age of 40

In Sweden much work has also been devoted to the study of enteric, Coxsackie and ECHO viruses Especially their connection with outbreaks of aseptic meningitis has been investigated

SWITZERLAND

Prof Dr O Gsell

Fur die Schweiz ist die epidemische Poliomyelitis heute die wegen ihrer Dauerschaden gefahrlichste jahrlich wieder auftretende Infektionskrankheit Die von der Schweizerischen Vereinigung gegen die Poliomyelitis und vom Eidg Gesundheitsamt gemachten Erhebungen ergeben folgende Resultate

MORBIDITAT

Die Poliomyelitis in der Schweiz ist seit 1930 in stetem Zunehmen begriffen Die Zahl der gemeldeten Falle betrug in den Jahren 1926-1930 im Durchschnitt 3 7 Falle pro 100 000 Einwohner in den Jahren 1951 1955 erreichte der Durchschnittswert bereits 19 6 Falle pro 100 000 Einwohner (absolute Zahlen 1954 1 678 1955 919 1956 973)

MORTALITAT

Die Sterbefalle an Poliomyelitis haben in den letzten 30 Jahren ebenfalls deutlich zugenommen jedoch nicht im gleichen Umfang wie die gemeldeten Falle Dieser Umstand ist auf die Tatsache zuruckzufuhren dass im letzten Jahrzehnt vermehrt aparalytischen Fallen gemeldet wurden Die durchschnittliche Mortalitat an Poliomyelitis in den Jahren 1926-1930 betrug 0 16 in den Jahren 1951 1955 jedoch bereits 1 66 pro 100 000 Einwohner (absolute Zahlen 1954 114 1955 60 1956 59)

LETALITAT

Die Letalitat d h das Sterberisiko den an Poliomyelitis erkrankten Personen hat sich von 21 8 in den Jahren vor 1930 auf 7 / in den letzten 3 Jahren vermindert Dieser scheinbare Ruckgang der Letalitat ist fast ausschliesslich durch das bessere Meldewesen und durch das Melden von aparalytischen Fallen bedingt

ALTERSVERTEILUNG

Die Altersverteilung der Krankheits- und Sterbefalle fur die Jahre 1951 1955 war die folgende

von den an Poliomyelitis erkrankten Personen waren

55 6 / Kinder unter 10 Jahren
23 7 Jugendliche von 10 19 Jahre
20 7 / Erwachsene uber 20 Jahre

von den an Poliomyelitis gestorbenen Personen waren

40 0 weniger als 10 Jahre alt
30 4 / zwischen 10 19 Jahre und
29 6 / 20 Jahre alt und mehr

GEOGRAPHISCHE VERTEILUNG

Die Verteilung der Poliomyelitisfalle innerhalb der Schweiz war sehr ungleich Auffallig ist eine hohe Morbiditat in der Ostschweiz und im Mittelland und die relativ geringe Morbiditat in der Westschweiz im Alpengebiet und in der Sudschweiz. Es wurde festgestellt dass im allgemeinen in den Stadten weniger Krankheitsfalle auftreten als in den umliegenden landlichen Bezirken Die grossten Morbiditatsunterschiede bestehen zwischen dunn und dicht besiedelten Landkantonen In den letzteren uberschritt der Durchschnittswert der letzten 5 Jahre 25 Falle/100 000 Einwohner

VIRUSISOLIERUNGEN

Virusisolierungen in Genf (Dr Bonifas) in Bern (Dr Krech) in Zurich (Dr Lindemann) zeigen eine deutliche Pradominanz des Typus 1 des Poliomyelitisvirus Bei den an Poliomyelitiserkrankten und Kontaktpersonen findet man am meisten Typ-1 Virus in Stuhl nur in einem kleinen Prozentsatz Typ 2 und Typ 3 Unter 279 isolierten Poliomyelitisstammen aus dem Stuhl durch Krech (1955 und 1956 fand sich Typ 1 in 90,3 / Typ 2 in 5 0 Typ 3 in 4 7 /)

ANTIKORPER GEGEN POLIOMYELITIS

Zur Beurteilung der Seuchenlage sind serologische Unter suchungen auf Abwehrkorper unerlasslich Es wurden in der Schweiz an verschiedenen Orten derartige Untersuchungen in grossem Ausmasse durchgefuhrt Die Ergebnisse welche in der wissenschaftlichen Ausstellung tabellarisch dargestellt sind zeigen dass in Gemeinden die seit 1950 frei von Poliomyelitis fallen geblieben sind ein grosser Prozentsatz der Kinder keine Antikorper gegen Typ 1 Poliovirus aufweisen In Gemeinden die viel Falle seit 1950 zu verzeichnen haben erreicht die Durchseuchung der Jugendlichen von 5 7 Jahren bereits Werte von uber 60 / jedoch nur gegen Typ 1 Ganz allgemein haben bis zum 7 Altersjahr nur wenige Prozent der Kinder Antikorper gegen alle 3 Typen Die serologischen Untersuchungen unterstutzen die Annahme dass Typ 1 als hauptsachlicher Erreger paralytischer Erkrankungen in Frage kommt wahrend die Typen 2 und 3 in der Bevolkerung fast ebenso haufig serologisch nachgewiesen aber bei Erkrankten weit seltener als Typ 1 gefunden werden

SCHUTZIMPFUNG

Da durchschnittlich weniger als 50 / der Kinder unter 10 Jahren und weniger als 75 /

La proportion de decès pour 100 malades montre la gravite de l epidemie de 1956 La frequence elevee de ces deces en milieu urbain comparativement au milieu rural tient en grande partie au nombre plus eleve des malades de plus de 15 ans en milieu urbain

Avant de terminer ce court expose je voudrais mentionner quelques resultats de nos examins effectues à l Institut Dr J Cantacuzene sur plus de 550 feces provenant de malades ou de leur entourage pour la mise en evidence du virus et la determination des types

Nous avons isole 114 ouches dont

110 Type 1	(96 49%)
1 Type 2	(0 87%)
3 Type 3	(2 64%)

Nous n avons jamais rencontre la coexistence avec les virus Coxsackie

Je me permets en terminant ce rapport de feliciter et remercier au nom de notre Gouvernement l Association Nationale pour la Poliomyelite des Etats Unis d Amerique pour l organisation de cette quatrieme Conference et d exprimer la gratitude de notre pays pour l hospitalite qui nous a ete offerte par la Suisse

SWEDEN

Dr Gunnar Olin

A severe outbreak of polio occurred in Sweden in 1953, with 4 7 paralytic cases per 100 000 during the epidemic year from April 1953 through March 1954 Since then the polio incidence has been low and slowly decreasing In 1954 there were only 5 cases per 100 000 in 1955 4 cases in 1956 3 5 cases per 100 000

The big outbreak of 1953 offered extensive opportunities for clinical studies of the disease and for investigations concerning its treatment The Stockholm hospital for epidemic diseases has made an important contribution in this field

Much interest has also been devoted to the problem of vaccination against polio by a team in Stockholm A Swedish polio vaccine produced from virus cultivated on human embryonic tissue was tried on 2 000 children early in 1955 No untoward reactions were observed However the Cutter incident as well as findings in Swedish control tests necessitated improvement of the safety of the vaccine It has also been considered important to try to increase the immunizing capacity of the vaccine Owing to the work connected with the solution of these problems, large scale production has been retarded

Therefore the first immunization program had to be carried out mainly with vaccine from the U S A released by courtesy of the federal authorities When the immunizations started in February of this year only about 100 liters of Swedish vaccine produced from virus cultivated on monkey tissue were available The program included children aged 4 to 11 years About 900 000 children were offered vaccination free of charge about 700 000 accepted and by now have received 2 inoculations each of 1 ml 3 weeks apart The program has been accomplished without an accident

The immunizing capacity of the vaccines used has been titrated both in children and in guinea pigs The result is presented at the Scientific Exhibit of the Conference

Since more Swedish vaccine became available in May it has been possible to extend the vaccination to certain categories at risk namely the staffs of virus laboratories and hospitals for epidemic diseases pregnant women and on a smaller scale people aged below 35 at their own expense The conscripts on duty in the armed forces have also been vaccinated

By extensive serologic investigations it has been shown that the sero immunity against poliomyelitis is developed rather slowly in the younger age groups in Sweden seronegative individuals are found even in the higher ages Therefore vaccination is needed up to the age of 40

In Sweden much work has also been devoted to the study of enteric Coxsackie and ECHO viruses Especially their connection with outbreaks of aseptic meningitis has been investigated

SWITZERLAND

Prof Dr O Gsell

Fur die Schweiz ist die epidemische Poliomyelitis heute die wegen ihrer Dauerschaden gefahrlichste jahrlich wieder auftretende Infektionskrankheit Die von der Schweizerischen Vereinigung gegen die Poliomyelitis und vom Eidg Gesundheitsamt gemachten Erhebungen ergeben folgende Resultate

MORBIDITAT

Die Poliomyelitis in der Schweiz ist seit 1930 in stetem Zunehmen begriffen Die Zahl der gemeldeten Falle betrug in den Jahren 1926 1930 im Durchschnitt 3 7 Falle pro 100 000 Einwohner in den Jahren 1951 1955 erreichte der Durchschnittswert bereits 19 6 Falle pro 100 000 Einwohner (absolute Zahlen 1954 1 678 1955 919 1956 973)

MORTALITAT

Die Sterbefalle an Poliomyelitis haben in den letzten 30 Jahren ebenfalls deutlich zugenommen jedoch nicht im gleichen Umfang wie die gemeldeten Falle Dieser Umstand ist auf die Tatsache zuruckzufuhren dass im letzten Jahrzehnt vermehrt aparalytischen Fallen gemeldet wurden Die durchschnittliche Mortalitat an Poliomyelitis in den Jahren 1926-1930 betrug 0 16 in den Jahren 1951 1955 jedoch bereits 1 66 pro 100 000 Einwohner (absolute Zahlen 1954 114 1955 60 1956 59)

LETALITAT

Die Letalitat d h das Sterberisiko den an Poliomyelitis erkrankten Personen hat sich von 21 8 in den Jahren vor 1930 auf 7 / in den letzten 3 Jahren vermindert Dieser scheinbare Ruckgang der Letalitat ist fast ausschliesslich durch das bessere Meldewesen und durch das Melden von aparalytischen Fallen bedingt

ALTERSVERTEILUNG

Die Altersverteilung der Krankheits und Sterbefalle fur die Jahre 1951 1955 war die folgende

von *den an Poliomyelitis erkrankten* Personen waren

55 6 Kinder unter 10 Jahren
23 7 Jugendliche von 10 19 Jahre
20 7 / Erwachsene uber 20 Jahre

von den an Poliomyelitis gestorbenen Personen waren

40 0 / weniger als 10 Jahre alt
30 4 zwischen 10 19 Jahre und
29 6 20 Jahre alt und mehr

GEOGRAPHISCHE VERTEILUNG

Die Verteilung der Poliomyelitisfalle innerhalb der Schweiz war sehr ungleich Auffallig ist eine *hohe Morbiditat in der Ostschweiz und* im Mittelland und die relativ geringe Morbiditat in der Westschweiz im Alpengebiet und in der Sudschweiz Es wurde festgestellt, dass im allgemeinen in den Stadten weniger Krankheitsfalle auftreten als in den umliegenden landlichen Bezirken Die grossten Morbiditatsunterschiede bestehen zwischen dunn und dicht besiedelten Landkantonen In den letzteren uberschritt der Durchschnittswert der letzten 5 *Jahre 25 Falle/100 000 Einwohner*

VIRUSISOLIERUNGEN

Virusisolierungen in Genf (Dr Bonifas) in Bern (Dr Krech) in Zurich (Dr Lindemann) zeigen eine deutliche Pradominanz des Typus 1 des Poliomyelitisvirus Bei den an Poliomyel*itiserkrankten und Kontaktpersonen findet man* am meisten Typ-1 Virus in Stuhl nur in einem kleinen Prozentsatz Typ 2 und Typ 3 Unter 279 isolierten Poliomyelitisstammen aus dem Stuhl durch Krech (1955 und 1956 fand sich Typ 1 in 90,3 / Typ 2 in 5 0 Typ 3 in 4 7 /)

ANTIKORPER GEGEN POLIOMYELITIS

Zur Beurteilung der Seuchenlage sind serologische Untersuchungen auf Abwehrkorper unerlasslich Es wurden in der Schweiz an verschiedenen Orten derartige Untersuchungen in grossem Ausmasse durchgefuhrt Die Ergebnisse welche in der wissenschaftlichen Ausstellung tabellarisch dargestellt sind zeigen dass in Gemeinden die seit 1950 frei von Poliomyelitis fallen geblieben sind ein grosser Prozentsatz der Kinder keine Antikorper gegen Typ 1 Poliovirus aufweisen In Gemeinden de viel Falle seit 1950 zu verzeichnen haben erreicht die Durchseuchung der Jugendlichen von 5 / *Jahren* bereits Werte von uber 60 / jedoch nur gegen Typ 1 Ganz allgemein haben bis zum 7 Altersjahr nur wenige Prozent der Kinder Antikorper gegen alle 3 Typen Die serologischen Untersuchungen unterstutzen die Annahme dass Typ 1 als hauptsachlicher Erreger paralytischer Er*krankungen in Frage kommt wahrend die* Typen 2 und 3 in der Bevolkerung fast ebenso haufig serologisch nachgewiesen aber bei Erkrankten weit seltener als Typ 1 gefunden werden

SCHUTZIMPFUNG

Da durchschnittlich weniger als 50 / der *Kinder unter 10 Jahren und weniger als 75 /*

der Jugendlichen von 10 19 Jahren Antikorper gegen Poliomyelitisvirus Typ 1 besitzen ist es in der Schweiz empfehlenswert bei allen Jugendlichen Schutzimpfungen vorzunehmen Die Tatsache dass Dreiviertel der Poliomyelitis patienten der letzten 5 Jahre Jugendliche unter 20 Jahren waren spricht zwingend fur eine Impfprioritat dieser Bevolkerungsgruppe

Die Impfungen mit der Salk Vakzine sind ab Ende 1956 in grossem Umfange durchge fuhrt worden und zwar nach Impfprogrammen die in den einzelnen Kantonen betrachtlich variierten ganz allgemein aber die Altersklassen zwischen 2 und 14 Jahren berucksichtigten Die Impfungen erfolgten zum Teil gratis zum Teil mit partieller finanzieller Beteiligung des Impflings Oeffentliche Impfaktionen waren stets freiwillig und ergaben eine Teilnahme zwischen 50 und 90 / in den fur die Impfung vorge sehenen Altersklassen

An Poliomyelitisvakzine wurde bis 15 Mai 1957 eingefuhrt 1764 Lit r und in der Schweiz selbst hergestellt 52 5 Liter Dieser Impfstoff reichte aus um mehr als 850 000 Personen zweimal zu impfen Man kann annehmen dass bis Sommer 1957 58 / aller Jugendlichen unter 18 Jahren in der Schweiz zweimal geimpft wurden Ueber die Ergebnisse kann erst 1958 berichtet werden

HILFE FUR DIE POLIOMYELITISGESCHADIGTEN

Fur die schweren Atmungslahmungen bei Poliomyelitis stehen in der Schweiz heute 130 moderne Respiratoren zur Verfugung darunter 30 Engstrom Apparate In den verschiedenen Klinikzentren sind Equipen zur Behandlung von Atmungsgelahmten geformt worden Am 1 April 1957 fanden sich 54 Kranke die total oder zeitweise auf Apparate angewiesen waren Fur die Behandlung im akuten Stadium wie auch fur die sachgemasse systematische Nachbe handlung fanden Kurse durch die Schweize rische Vereinigung gegen die Poliomyelitis statt. Es stehen eine Reihe besonderer orthopadischer Institute und Badekurorte zur Verfugung

Gegen die materiellen Folgen der Kinder lahmung sind heute 2 8 Millionen Personen (bei rund 5 Millionen Einwohnern der Schweiz) im Schweiz Verband fur die er weiterte Krankenversicherung sowie eine wei tere betrachtliche Zahl bei zwei privaten Ver sicherungsgesellschaften speziell gegen Kinder lahmungsfolgen versichert, mit einer Entschadi gung zwischen 20 50 000 Franken abgestuft nach Altersgruppen In einzelnen Kantonen besteht eine besondere Invalidenversicherung in Bearbeitung ist eine schweizerische Invalidi tatsversicherung Fur die Wiedereingliederung der Gelahmten kummern sich speziell zwei grosse Organisationen Pro Infirmis und Schweiz Arbeitsgemeinschaft zur Einglieder ung Behinderter in die Volkswirtschaft

STATISTISCHE ANGABEN

Detaillierte Zahlenangaben uber das Vorkom men der Poliomyelitis in der Schweiz und uber die Verteilung des Antikorpergehaltes in der Bevolkerung auf die verschiedenen Kantone uber Institutionen und Versicherungen finden sich in der wissenschaftlichen Ausstellung auf 27 Tafeln zusammengestellt mit Hilfe der Schweiz Vereinigung gegen die Poliomyelitis und des Eidg Gesundheitsamtes

THAILAND

Dr C Purananda

The disease was first recognized about 30 years ago when the Medical School was reorgan ized with the aid of the Rockefeller Foundation Since then a few cases among children were reported each year The diagnosis was made on the history of the illness and the symptom of paresis which was temporary complete recovery following after a few months There was no mortality therefore it was considered to be a mild infection like coryza After World War II the number of immigrants from Europe and America increased and formed an important colony in the capital of Thailand In 1950 and 1951 cases of poliomyelitis were *reported* in Singapore which is one of the nearest ports con nected to Bangkok by sea rail and air At tempts were made to safeguard the children and the foreign communities and no cases were reported in Bangkok

In September 1952 the first case of polio myelitis was reported in a Swiss lady who died after 4 days of illness A few days later a Danish lady who was expecting a child contracted the disease She was rushed to Singapore and was placed in an iron lung until the birth of her son Between September and December 1957 there

were 388 cases reported in Bangkok This included all age groups foreigners as well as Thai with a mortality of 6 per cent Diagnosis was made by clinical examination and the treatment was symptomatic An iron lung was acquired in the latter part of the epidemic When the epidemic was over there were quite a few cases of paralysis but no cases of spinal column involvement

It is considered that this was the first epidemic of poliomyelitis in Thailand A few epidemologists tried to find reasons for it and came up with the following hypotheses

1 The improvement of sanitary conditions in our country
2 The introduction of new strains
3 The transmission of old strains into a new fertile soil

The improvement of sanitary conditions was ruled out by the fact that there were no remarkable improvements since the war Some public utilities were not as good as in the pre war years therefore the inhabitants still stand a good chance of contracting small repeated infections

As for the second theory by sampling blood of aged inhabitants of a province situated 100 Km east of Bangkok where no cases of polio were reported during the epidemic it was found that the 3 types of polio virus already existed in Thailand[3] Samples of blood from children between the ages of 2 to 7 who were convalescing from this epidemic of polio were also tested The result was that nearly all of the samples showed antibody against Types 1 2 and 3 but antibody to Type 3 was less frequent[2] The results of these 2 analyses strengthen the theory that there is constant mild infection taking place in the people

The third hypothesis that of local strains being introduced into a fertile soil needs more experimentation to be proved

Attempts were made to establish a virus laboratory with facilities for making possible early diagnoses However these attempts have been in vain because of the shortage of all factors from virologists technicians and trained employees to equipment The latter is easier to acquire than the first three A few foreign organizations and institutions have promised aid but nothing has materialized as yet

The report of the success of Salk vaccine from the United States has aroused Thai medical scientific and educational workers to consider whether the use of Salk s vaccine is indicated in Thailand[4] A review of the report of the Conference of WHO Experts held in Stockholm in November 1955 together with the results of experiments on polio antibody in Thai adults and children show that Salk vaccine is not indicated Also since the epidemiology and the mode of infection of polio resembled typhoid fever there are a few cases of poliomyelitis yearly and if the epidemic cycle will be the same as in other countries we are expecting another outbreak in September of this year However as yet nothing has been done except the improvement of the water supply in Bangkok Information should be distributed to the public accordingly

It is of interest to compare countries where poliomyelitis is most dangerous with countries where the disease is less dangerous and with those countries were polio is not dangerous In the first group are the United States of America Scandinavia and Switzerland The second group is composed of most of the countries in Europe and Canada The third group comprises the Near East and the Far East countries The countries in the first group are those with the best sanitary conditions their people work very hard and the amount of sunshine through the year is not enough to attentuate the virus The countries in the second group have a comparatively lower standard of sanitary conditions their people work very hard and the amount of sunshine is comparatively less through the year In a few countries in this category there are other virus infections such as influenza The countries of the third group have poor sanitary conditions and a multitude of bacteria flourish thus hampering the thriving of polio virus[4] Their people are not hard working There is a lot of sunshine throughout the year the amount of ultraviolet in the sunshine attenuates the viruses[1] and they become less virulent

REFERENCES

1 Cruickshank R Ultraviolet light in the control of respiratory infection in schools *in* Proc Sixth Internat Congr Microbiol Springfield Ill Thomas 1953

2 Koprowski H Purananda C and Sringam S Antibodies in poliomyelitis conva

lescents in Bangkok J Microbiol Soc Thailand vol 1 no 1 1957
3 Preliminary report on the epidemiology of poliomyelitis in Thailand M J Chulalongkorn Hosp M School vol 2 no 1
4 Puranananda C The problem of antipolio vaccine in Thailand Siriraj Hosp Gaz vol 8 no 6 1956

UNION OF SOVIET SOCIALIST REPUBLICS

Dr Valentin D Soloviev

In the Soviet Union the marked increase in the incidence of poliomyelitis was first noted in 1955 when the morbidity rate reached a figure never previously observed namely 8 cases per 100 000 inhabitants Approximately the same figure was observed in 1956 In 1957 the incidence seems not to decline as we may judge by the available information

A summer autumn seasonal prevalence of *poliomyelitis was pronounced in 1955 and 1956* the peak of the incidence being in July August September and October

Distribution by age groups shows that young children under 7 are affected most often they make up about 85 per cent of cases

However in some western regions of the U S S R we see a different age group distribution In the Estonian Soviet Socialist Republic for instance about 15 to 20 per cent of cases were adults of 20 or more years of age

The average lethality of poliomyelitis in 1955 was 3 7 per cent and in 1956 2 6 per cent

Typing of the strains isolated in 1956 showed the following distribution of virus types 63 per cent of the strains belonged to Type 1 12 per cent to Type 2 and 25 per cent to Type 3 Moreover from stool specimens of patients and their contacts a number of strains were isolated which were untypable when tested with specific sera of all 3 types of polio virus

For the control of poliomyelitis two institutes have been established the Poliomyelitis Institute of the Academy of Medical Sciences of the U S S R aiming at research work in the problem and the Moscow Institute of the Ministry of Health of the U S S R mainly intended for the manufacture of Salk vaccine

Laboratories have been set up in large towns of the U S S R for the diagnosis of poliomyelitis

Great attention is given to treatment of poliomyelitis patients Besides special departments in children s hospitals there are centers for rehabilitation therapy and special sanatoria

The treatment of poliomyelitis patients is free of charge as are all medical services in the U S S R

UNITED KINGDOM (Colonies)

Dr W H Bradley

The available evidence indicates that poliomyelitis must be regarded as being endemic in practically all the British Colonial Territories overseas In the majority of the territories the incidence of paralytic cases is sporadic but outbreaks of epidemic type and proportions occur in circumstances which at present are unpredictable Reference was made to some of these at the Third International Conference Several *have occurred since that date and are referred* to here under the name of the individual territory affected

The precise immunologic state of the population of the overseas territories is not known Evidence derived from studies of the incidence of the disease indicates that a fair degree of immunity is acquired by the local communities by the age of 5 years but that among immigrants from Europe and the western countries older children and young adults are susceptible and among them the incidence rate is disproportionately high In the Kenya epidemic of 1954 the racial incidence rates per 100 000 were *Europeans 254 Asians 46 5 Africans 6.5* These figures are fairly typical of racial incidence in most of the African Colonial Territories

Pilot serologic surveys have been carried out in several territories The results reported from Lagos Nigeria showed that of 26 sera examined only 1 a child of 2 years was negative for all 3 types In Singapore 40 per cent of children of local origin in the 5 to 9 age group had antibodies to all 3 types Similar findings have been reported from Uganda In the Far East an extensive survey sponsored by the World Health Organization is being carried out by Professor Hale of the University of Malaya This will include Hong Kong The Federation of Malaya

Singapore Borneo and Sarawak and the work when completed will give a much more accurate picture than can be deduced at present.

Jamaica The epidemic which started in June 1954 was fading out early in 1955 In a population of approximately 1,500 000 792 cases were notified up to February 5 1955 In 1956 the incidence had returned to endemic proportions This was the first serious epidemic reported in the island

Trinidad There is a record in this island of several epidemics in recent years 1937 106 cases 1941 59 cases 1942 135 cases 1945 99 cases In 1954 an epidemic coincided with that in Jamaica 186 cases were notified of which 50 per cent were under 5 years of age and 163 under 16 years There are indications of another outbreak occurring in 1957 but full details are not yet available

Tanganyika Poliomyelitis has occurred in epidemic form as follows in 1954 170 cases 1955 123 cases 1956 458 cases A feature of these outbreaks in this large territory with a very low population density has been the localized nature of the outbreaks In 1956 there were 3 distinct areas affected without a general spread to the larger urban centers This outbreak was continuing in 1957

Vaccination programs are being planned in many territories and in some have already been developed on a considerable scale The full development of vaccination programs obviously must be influenced by the availability of vaccine and internal economy The accessibility of low density populations is also a controlling factor Apart from these practical considerations it is realized that more precise information is required as to the immunologic state of communities and age groups It is felt that this will require further investigation before anything approaching mass vaccination campaigns can be justified scientifically Meanwhile programs are being framed to offer protection to those who can reasonably be assumed to be at greatest risk

URUGUAY

Prof Enrique M Claveaux

La poliomielitis en el Uruguay diagnosticada desde 1905 se ha mantenido presente desde esa fecha hasta ahora en forma de brotes anuales de intensidad variable Los empujes más importantes fueron los de los anos 1936-1937 con 284 casos 1943 con 167 casos 1945 con 165 casos y 1954 1955 con 574 El ultimo empuje configuro una verdadera epidemia siendo evidente que la enfermedad ha adquirido ya una importancia epidemiológica alarmante

La enfermedad predomina en los meses calurosos del ano Excepcionalmente aparece en el invierno como ha ocurrido en la ciudad de San Jose (2? casos entre Abril y Septiembre)

Las estadisticas del pais consignan solo las formas paraliticas de la enfermedad En la ultima epidemia la morbilidad fué de 20 por 100 000 habitantes

La mortalidad en esa misma epidemia fue de 6 7 %

La mayor morbilidad correspondio a ninos hasta los 3 anos (44 4)

La morbilidad para mayores de 14 anos represento el 14 3 %

Las formas respiratorias (11) tuvieron una mortalidad de 55 0 %

Las formas graves respiratorias y paralisis extendidas representan 50 % del total

El país ha realizado en los tres ultimos anos un esfuerzo marcado en la lucha contra la paralisis infantil Se ha organizado un servicio autonomo dedicado exclusivamente al tratamiento de las formas agudas Se dispone de 4? pulmones de acero y 4 aparatos de presion positiva (Engstron) utilizandose además bas ulantes y corazas El control de la capacidad vital y aire corriente se hace en forma satisfactoria con aparatos adecuados

Los servicios complementarios de rayos X laboratorio y fisioterapia atendidos por especialistas funcionan eficientemente

El publico ha respondido al llamado de las autoridades y sigue contribuyendo ampliamente para la instalacion de servicios de recuperacion

La vacunacion por el metodo Salk actualmente se aplica en el pais en forma voluntaria y en las condiciones técnicas universalmente admitidas como de rigor

Se practica en tres inyecciones—las dos primeras a un mes de distancia entre ellas y la tercera al 6 mes de la segunda

La vacuna ha sido en general bien tolerada

El numero actual de vacunados es de 63 466 con la primera dosis y de 50 408 con 2 dosis

Se ha vacunado preferiblemente a ninos entre 6 meses y 6 anos de edad (46 911) de los cuales

38 000 recibieron tambien la segunda dosis

La vacunacion realizada fuera de periodo epidemico no ha podido traducirse en hechos *que permitan juzgar su eficiencia*

En lo que va transcurrido del presente ano solo se han producido en el Uruguay 25 casos de paralisis Dos de estos casos estaban vacunados uno con una dosis y otro con dos dosis

Aun no se ha comenzado a aplicar la tercera inyeccion

Consideramos que un juicio sobre la calidad de los resultados obtenidos por la vacunación solo podria en nuestro pais realizarse cuando hayamos vacunado toda la poblacion infantil con las tres dosis como la vacunacion no es obligatoria dependera en gran parte de la aceptacion del publico poder llegar a aquel desideratum

Creemos que si la inmunidad tiene caracter precario (corta duracion) la necesidad de repetir las inoculaciones alejará al publico de los dispensarios Si se agrega a esto el caracter parcial de la vacunacion no obligatoria estimamos que sera muy dificil que por el metodo Salk se logre hacer desaparecer la paralisis infantil en nuestro pais

YUGOSLAVIA

Prof K Todorovitch

Pendant longtemps jusqu au 1928 la poliomyelite en Yougoslavie existait a l état sporadique Jusque la on n avait note que des cas isoles tant chez des enfants que chez des adultes Jusqu en 1940 le nombre des malades etait petit moins de 100 par an excepte l annee 1928 ou le nombre des malades atteignit le chiffre de 201 Pendant cette periode la poliomyélite etait accompagnee d une mortalite tres élevee laquelle certaines annees atteignait meme 21 % (1929) 23 % (1940) 30 % (1934) et 33 % (1929) Le nombre des malades par rapport au nombre des cas mortels etait en proportion inverse

A partir de 1946 le nombre des malades atteints de la *poliomyelite va en augmentant* sans quelque regularite Au cours des 10 dernieres annees le nombre etait en moyenne 2 fois plus grand qu au cours des 2 periodes de 10 ans precedentes demontrant meme en certaines annees des augmentations notables comme cela fut le cas par exemple en 1953 quand 715 cas furent enregistres dont 41 etaient mortels (mortalite = 5 73 %) Au cours des 2 annees suivantes l on enregistra un nombre 2 fois moins grand de malades de la poliomyelite soit 339 en 1954 avec mortalite de 7 96 % et 375 en 1955 avec mortalite de 4 31 % L annee passee en 1956 le nombre des cas enregistrés en Yougoslavie a atteint le chiffre de 1254 dont 53 avaient ete mortels (lethalite = 4 72 %)

Au cours des 30 dernières années la poliomyelite en Yougoslavie se manifestait presque toujours a l etat sporadique et ce n est que certaines annees qu elle prenait un caractere d epidemie mais toujours faible d envergure

Le tableau 19 indique le repartition des malades par republique telle qu elle se présentait en 1956 annee au cours de laquelle fut noté le plus grand nombre de cas

La poliomyelite ne se manifestait que dans certaines regions delimitées sans qu il y ait eu tendence a sa propagation par contacts et sans qu en ces endroits le nombre de malades avec des paresies ou des paresthesies manifestes ait ete note au sein des memes collectivites par exemple au sein de la meme famille parmi les eleves d une meme classe parmi les habitants d une meme rue etc

Au point de vue mortalité la Yougoslavie se trouve parmi les pays ou la mortalite par suite de la poliomyelite est des plus faibles

Tableau 19 Repartition des Malades par République 1956

République Populaire	Nombre d Habitants	Malades	Cas Mortels
Serbie	7 335 000	754	22
Croatie	4 043 000	112	4
Slovénie	1 516 000	260	24
Bosnie et Herzegovine	3 084 000	67	1
Macédonie	1 417 000	56	2
Montenégro	458 000	5	—

Comme dans les autres pays européens la poliomyélite en Yougoslavie a le caractère de maladie saisonnière Au cours des derniers mois de l'automne au cours de l'hiver et du printemps le nombre des malades était très petit et n'augmentait sensiblement que pendant l'été Cette régularité saisonnière a été notée indépendamment du nombre des malades

La plupart des malades en Yougoslavie sont des enfants âgés de 1 à 3 ans Pour les nourrissons le nombre des cas était toujours légèrement inférieur Dans les régions où la poliomyélite apparaît comme maladie épidémique la mortalité est légèrement supérieure chez les adolescents de 14 à 15 ans à celle chez les enfants plus jeunes ou chez les adultes En 1956 année où fut noté le nombre de cas le plus grand le malade le plus jeune était un nouveau-né de 2 semaines et le plus âgé un homme de 56 ans

La plupart de nos malades provenaient des campagnes (1 6 1 0) L'on n'a pas pu établir de lien épidémiologique convaincant entre différentes agglomérations éloignées ou proches ni la source commune possible de l'infection ni la même voie ou manière de la diffusion de virus

L'analyse des cas qui se sont manifestés uniquement sur un territoire sur lequel vivent 5 000 000 d'habitants classés d'après les dates de la maladie démontre une dispersion telle qu'elle ne permet d'établir entre eux aucun lien quelque douteux qu'il soit Même dans les grandes villes et agglomérations de 50 à 100 000 habitants ou dépassant 500 000 ce n'est relativement que très rarement qu'on a pu noter l'apparition de la maladie le même jour chez un nombre plus grand de personnes sur un territoire donné L'année passée la maladie avait au cours de la période de l'été la caractère d'épidémie dans certaines agglomérations industrielles Toutefois même à cette occasion l'apparition de la maladie chez 2 ou plusieurs personnes appartenant au même établissement n'était qu'exceptionnelle

Dans les localités où la poliomyélite avait pris des proportions épidémiques l'on n'a pu chez les animaux domestiques relever aucune autre ou semblable maladie de caractère sporadique ou épizootique

Il y a lieu de mentionner que l'apparition en plus grand nombre de la poliomyélite dans certaines régions de Yougoslavie avait été précédée généralement chez les enfants et adolescents d'affections fébriles de courte durée avec des signes manifestes de méningite et de méningo-encéphalite et avec de l'albuminorachie et pléocytose modérées dans le liqueur L'affection se passait sans leucocytose dans le sang et sans qu'en ait pu être relevée la cause parmi les microbes pathogènes connus Les recherches virusologiques sont restées sans résultats

Lors de l'épidémie qui a eu lieu dans la République de Serbie on a noté un grand pourcentage de malades chez lesquels s'était manifestée la paralysie du N facialis soit comme l'unique soit comme l'un des signes accompagnant les lésions nerveuses périphériques chez des cas indubitables de poliomyélite La proportion était de 2 3 (41 63)

Des recherches virologiques d'envergure limitée ont démontré qu'en Serbie par exemple sur 78 virus identifiés les 2/3 appartenaient au type 1 environ 7 / au type 2 et ¼ au type 3

Les recherches sérologiques menées sur un territoire limité ont démontré que les anticorps pour les type 2 se trouvent chez les enfants de moins de 2 ans dans une proportion de 60 chez les enfants de 4 ans—80 et chez les adolescents et les adultes presqu'à 100

D'après ces résultats on dirait que les enfants des campagnes démontrent plus lentement et plus tard la présence d'anticorps dans le sang que les enfants des villes

Les anticorps pour le type 3 du virus polio apparaissent plus tard et plus lentement

Les anticorps pour le type 1 sont les moins représentés

Note The Reports published are those for which requested texts were received

Vaccination Against Poliomyelitis

MONDAY AFTERNOON, JULY 8, 1957

Chairman

DR J ØRSKOV
Statens Seruminstitut
Copenhagen

Speakers

DR JONAS E SALK
Virus Research Laboratory
University of Pittsburgh
Pittsburgh

DR ALEXANDER D LANGMUIR
Epidemiology Branch
Communicable Disease Center
Atlanta

DR DAVID BODIAN for the Technical Committee on Poliomyelitis Vaccine
USPHS
School of Hygiene and Public Health
Johns Hopkins University
Baltimore

Discussants

Moderator DR THOMAS FRANCIS JR
Department of Epidemiology
University of Michigan
School of Public Health
Ann Arbor

DR P L BAZELEY
Commonwealth Serum Laboratories
Department of Health
Melbourne

DR SVEN GARD
Karolinska Institute
Stockholm

DR JAMES H S GEAR
South African Institute for
Medical Research
Johannesburg

PROF DR RICHARD HAAS
University of Freiburg
Freiburg

PROF DR CURT HALLAUER
Institute of Hygiene and Bacteriology
Berne

DR A BRADFORD HILL
Department of Medical Statistics
and Epidemiology
London *School of Hygiene and*
Tropical Medicine
London

DR HERDIS von MAGNUS
Statens Seruminstitut
Copenhagen

DR RODERICK MURRAY
Division of Biologics Standards
National Institutes of Health
Bethesda Md

DR KARI PENTTINEN
State Serum Institute
Helsinki

PROF DR HEINRICH PETTE
Neurologische Universitats Klinik
Hamburg Eppendorf

DR JOSEPH E SMADEL
National Institutes of Health
Bethesda Md

PROF DR C H STUART HARRIS
Department of Medicine
Royal Hospital
Sheffield

DR J O H TOBIN
Medical Research Laboratories Council
Holly Hill Hampstead
London

Basic Principles Underlying Immunization Against Poliomyelitis with a Noninfectious Vaccine

DR JONAS E SALK

At the International Poliomyelitis Congress in Rome in 1954 a summary was presented of information then available relevant to vaccination with a killed virus vaccine For this Congress a similar effort is being made but as is to be expected the questions before us are different from those of 3 years ago Principal interest now centers around vaccine effectiveness on the practical questions of the degree and the duration of immunity and the more fundamental questions concerning the mechanisms of immunity

In considering which parts of the whole to discuss and how best to present the pertinent facts and the inferences that have been drawn therefrom it seemed to me that we might begin by making an admittedly erroneous supposition namely that all humans possess exactly the same number of antibody forming cells and that these cells are all equally responsive immunologically

The first example is that of a child in whom the antibody persistence pattern after primary immunization is as illustrated in Figure 32 If all children similarly treated responded in this way, it would be generally agreed that they would be immune to paralysis at least for the period of time here indicated and that is 3 years Figure 33 depicts a child in whom reinoculation was performed 7 months after primary vaccination and the same inference in regard to persistence of immunity to paralysis could be drawn in this instance and that is 4 years

Additional examples of persistence of antibody over a 3½ year period are illustrated in Figure 34 The 2 children used for illustration were vaccinated first in May 1953 and a secondary or booster dose was given in December 1953 The results of serologic tests on 9 blood samples are shown The first 7 black symbols in the case of child F 297 on the left represent the results of simultaneous serologic testing The seventh blood sample was retested along with No 8 when the latter was obtained at 2½ years and No 8 was retested along with No 9 when the latter was obtained at 3½ years The actual values for antibody titer obtained each time are plotted and the tendency for persistence of antibody for each type is clearly evident

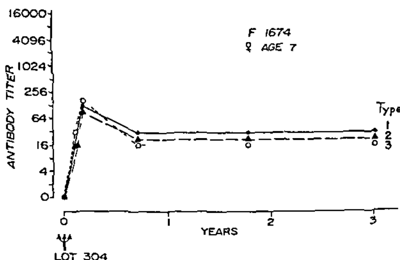


Fig 32 Persistence of antibody 3 years after primary vaccination

Table 20 Persistence of Type 1 Antibody Over 3½ year Period in Children Given Booster Dose at 7 or 11 Months after Primary

Subject Age in 1953	1½ Yrs	2½ Yrs	3½ Yrs
F 137	512	512	
13 yrs		16	16
F 328	256	128	
10 yrs		64	64
F 336	128	32	
12 yrs		128	32
F-45	128	32	
9 yrs		64	32
F 135	128	128	
11 yrs		16	32
F 182	32	16	
9 yrs		16	16
F 297	32	16	
9½ yrs		16	16
F 291	32	32	
11½ yrs		16	32
F 207	32	32	
8 yrs		32	32
F-47	32	32	
10 yrs		4	4
F 27	32	16	
5 yrs		16	32
F 136	16	8	
7 yrs		0	0
F 69	8	16	
16 yrs		4±	4±

Table 21 Persistence of Type 2 Antibody Over 3½ year Period in Children Given Booster Dose at 7 or 11 Months after Primary

Subject Age in 1953	1½ Yrs	2½ Yrs	3½ Yrs
F 136	256	32	
7 yrs		128	128
F-47	64	32	
10 yrs		32	32
F 27	64	32	
5 yrs		64	32
F 69	32	128	
16 yrs		256	64
F 328	32	64	
10 yrs		64	64
F-45	3	64	
9 yrs		3	32
F 297	16	16	
9½ yrs		8	8
F 135	16	8	
11 yrs		16	16
F 137	16	32	
13 yrs		8	8
F 182	16	8	
9 yrs		8	8
F 291	16	16	
11½ yrs		16	16
F 207	16	8	
8 yrs		8	8
F 336	8	16	
12 yrs		16	8

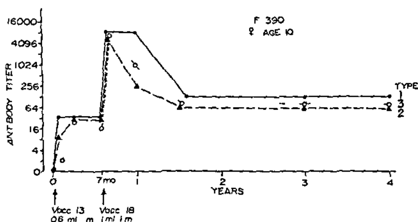


Fig 33 Degree of persistence of antibody over a 4 year period.

TABLE 22 PERSISTENCE OF TYPE 3 ANTIBODY OVER 3½ YEAR PERIOD IN CHILDREN GIVEN BOOSTER DOSE AT 7 OR 11 MONTHS AFTER PRIMARY

SUBJECT AGE IN 1953	1½ YRS	2½ YRS	3½ YRS
F 297	512	64	
9½ yrs		256	128
F 136	128	32	
7 yrs		32	32
F 135	32	16	
11 yrs		32	8
F 336	32	32	
12 yrs		16	16
F-47	32	32	
10 yrs		64	64
F 328	32	32	
10 yrs		64	64
F 27	16	32	
5 yrs		64	64
F-45	16	16	
9 yrs		16	16
F 137	16	8	
13 yrs		8	8
F 291	16	16	
11½ yrs		4	4
F 202	16	8	
8 yrs		8	8
F 69	8	16	
16 yrs		16	8
F 182	8	8	
9 yrs		±	4

Additional examples are shown in Tables 20 21 and 22 In Table 20 we show data for Type 1 The first line in each instance represents results of simultaneous tests for antibody in blood samples drawn 1½ and 2½ years The second line represents the results of the simultaneous tests for antibody in the 2½ and 3½ year bleedings The degree of comparison of results of the 2½ year blood tests on the two different occasions shows the degree of comparability that is obtained in repeat tests

Table 21 shows data of the Type 2 antibody levels and you will observe the tendency for there to be persistence between the 1½ and 2½ year points and the 2½ and 3½ year points in all instances I have been asked whether or not this might represent maintenance of immunity due to natural infection and I can tell you that in those instances where natural infection has occurred there is usually a rise of antibody to but one type Table 22 shows the degree of persistence of antibody to the Type 3 virus

Now from these evidences it seems likely that antibody will be demonstrable in most individuals for a number of years after an effective course of immunization Therefore the attainment of effective immunization initially is the point of first importance and the achievement of this seems to be dependent on the administration of a sufficient quantity of antigen It is

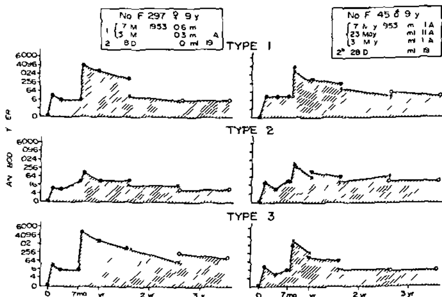


FIG 34 Degree of persistence of antibody over 3½ years



Fig 35 Dosage effect. Type 1 antibody response 2 weeks after 1 dose of Reference Vaccine A given intramuscularly to subjects with no demonstrable preantibody to any type (Salk J E J A M A 158 1239)

self-evident that a minimal number of antigenic units will engage merely a minimal number of antibody forming cells and the amount of antibody induced thereby will also be minimal A more potent antigen will induce the formation of higher levels of antibody not only because more cells are involved but also possibly because each cell is exposed to more antigen With more potent antigens saturation of the antibody inducing mechanism is more likely to be approximated as is probably the case in the infectious process where virus multiplication occurs and where a large number of antibody forming cells are engaged

Data in Figure 35 show that individuals given the same dose of antigen are more responsive than others From these data it also appears that the response of the individual can be enhanced if the amount of antigen administered is increased at least within limits And you can see clearly from Figure 35 when a large dose is given all respond to a single dose of vaccine and when a smaller dose of vaccine is given some fail to respond This dosage response effect can be demonstrated with Types 2 and 3 as well Thus it is clear that failure of response could result either because of an inadequate or impotent stimulus or because of a relatively poorly responding immunologic mechanism Therefore the problem seems to be one of adjusting vaccine potency and number and spacing of inoculations to effect sufficient stimulation especially in those individuals who are the least responsive immunologically

Although the level of antibody observed after vaccination is the resultant of (1) the degree of responsiveness of the individual (2) the amount of antigen administered (3) th spacing be

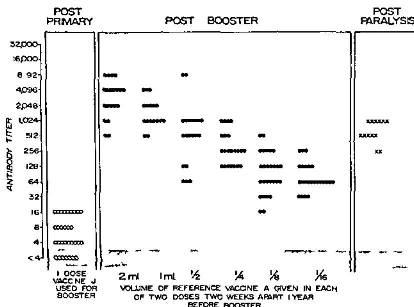


Fig 36 Type 1 antibody response after booster in relation to potency of vaccine used for primary inoculation and as compared with antibody titer following natural infection (Salk J E Am J Pub Health 47 1)

tween inoculations and (4) the time that has elapsed since the last inoculation it is abundantly clear that a demonstrable level of antibody once induced by a killed polio virus vaccine is not necessarily evanescent With the degree of biologic variation observed in antibody response induced by primary vaccination and in level of antibody present in intervals thereafter or following a booster dose it is clear that the practical problem requires that a sufficient excess of antigen be incorporated in the vaccine to induce the desired effect consistently If to the problem posed by human variation in responsiveness is added the variation in potency of vaccine preparations it is probable and it is also understandable that the full potential of the procedure has not yet been realized

Nevertheless decisions must continue to be made and goals must be defined The objective to be attained can be decided in part through tests made with a reference vaccine of reasonable potency against which unknown vaccines can then be standardized This we have attempted to do with Reference Vaccine A It has been demonstrated that there is a dosage response relationship not only with respect to the level of antibody induced after primary vaccination as may be seen from Figure 35 but also with respect to the level of antibody induced after secondary stimulation as is shown in Figure 36

Let me point out the nature of this experiment. Groups of children were given 2 doses consisting of 2 cc of vaccine 1 cc a half a quarter an eighth or a sixteenth 2 weeks apart, a year before a booster dose was given The antibody level was then determined 2 weeks after the booster dose The control group that had not been vaccinated previously and had received the booster dose of vaccine is shown on the left you will see the way in which those who receive a primary immunization with the different dosages responded to the same booster dose of vaccine that was equal in all groups You will see clearly that the degree of response to the booster is related to the quantity of antigen administered primarily Thus we can see that the degree of hyperreactivity induced by the primary immunologic experience is revealed by the degree of response at the time of revaccination

These findings indicate that the hyperreactivity is not an all-or none state but that it exists in varying degrees We shall return to this question in a moment

Data in Figure 37 indicate the degree to which antibody response was induced and maintained in a group of approximately 150

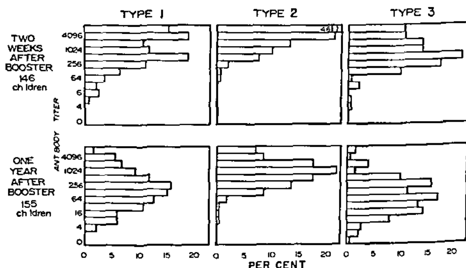


Fig 37 Antibody distribution 2 weeks and 1 year after booster Primary inoculation with commercial vaccine Primary 2 doses 1 cc i m 2 weeks apart 6 lots 1955 vaccine from 4 producers Booster 1 cc Vaccine J i.m 1 year later

children who possessed no antibody for any type before vaccination This figure illustrates antibody distribution and the length of the columns indicates the number of individuals in per cent who possess antibody at the different levels shown on the left hand scale 2 weeks after the booster dose in children who had received 2 doses of 1 cc intramuscularly 2 weeks apart of 6 lots of 1955 commercial vaccine from 4 producers A year later they received 1 cc of Vaccine J intramuscularly and the levels shown are those observed 2 w eks after the booster dose for Type 1 Type 2 and Type 3 You will observe that there are none in the zero range

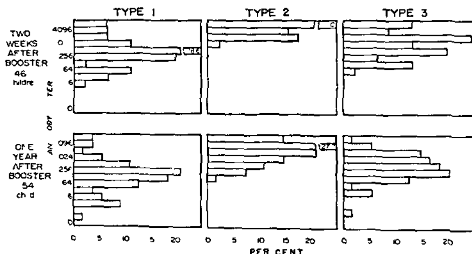


Fig 38 Antibody distribution 2 weeks and 1 year after booster Primary inoculation with Reference Vaccine A (composite for groups given 2 ml 1 ml or ½ ml) Primary 2 doses i m 2 weeks apart 2 cc 1 cc or 1 cc 1 ? dil Reference Vaccine A Booster 1 cc Vaccine J i m 1 year later

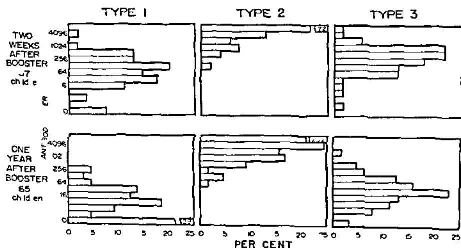


Fig 39 Antibody distribution 2 weeks and 1 year after booster Primary inoculation with Referen e Vaccine A (composite for groups given ¼ ml ⅛ ml or 1/16 ml) Primary 2 doses 1 cc i m 2 we ks apart 1 4 1 8 or 1 16 dil Reference Vaccine A Booster 1 cc Vaccine J i m 1 year later

and the majority of them are quite high One year later without any further inoculations antibody determinations were made and you will see the tendency for decline but you will also observe that there are few if any in the zero column Thus it is clear that there has been a considerable degree of persistence of antibody in those who received commercial vaccine of the 1955 vintage followed by laboratory vaccine for the booster dose

Figure 38 shows similar data for a group of children (the irregularities in the smoothness of the curve as compared to Fig 37 are due to the smaller size of groups) who had received either 2 cc 1 cc or one half cc of Reference Vaccine A followed by a booster 1 year later of Vaccine J

Here again you see 2 weeks post booster the high levels of antibody You see the tendency for decline but there is still antibody 1 year after vaccination in almost all The picture is somewhat different in Figure 39 which shows the results observed in a group of children who had had primary immunization with a smaller dose of Vaccine A a 1 4 1 8 or 1 16 dilution followed by the booster You will see that some of them did not respond to Type 1 even to the booster dose when their primary sensitization was with this level of antigen For Type 2 and Type 3 there was a fair response But at the end of 1 year there is a tendency for antibody levels to decline so that 29 per cent had no Type 1 antibody that was detectable in a group of 65 children The Type 2 levels were high and Type 3 was somewhat intermediate

Figure 40 shows a comparison between the group that received 6 lots of commercial vaccine and the group that received the larger dose of Vaccine A There is a much better response in those who received the commercial vaccine than those who received small doses of Reference Vaccine A The Type 2 levels were about equally high in all groups and the Type 3 showed a somewhat lower level

It is clear from these data that a measurable level of antibody does persist and that the degree of persistence of this state is influenced by the amount of antigen employed

But what degree of immunity are we seeking to establish? Do we wish to have a persistently demonstrable level of antibody or merely an effective hyperreactive immunologic mechanism? Let us consider such individuals as those in whom antibody levels were once induced (as with the small doses of Reference Vaccine A shown in the upper right hand frame of Fig 40) and in whom antibody is no longer demonstrable 1 year after the booster dose

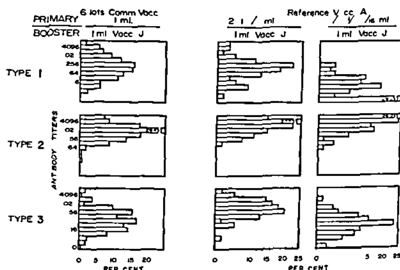


Fig 40 Influence of primary immunization on persistence of antibody Comparison of commercial vaccines with different doses of Reference Vaccine A

As an illustrative example Figure 41 indicates the degree of hyperreactivity demonstrable 7 months after primary immunization in one individual and about 1½ years after the last dose of vaccine in another The level to which antibody increases after inoculation in such individuals suggests the probability that there is an acceleration in the appearance of high levels of antibody And this brings to mind MacLeod s explanation for long lasting immunity to certain infectious diseases This is illustrated in Figure 42 and many of you have seen this in MacLeod s paper and in some of our publications

As applied to polio the expectation is that it is a disease with a long incubation period and therefore the secondary response comes in before the disease process would ensue As applied to polio in immunologically experienced persons a sufficiently rapid output of antibody in the interval between inception of infection and invasion of or spread within the central nervous system (CNS) could provide the basis for a state of immunity that might be expected to last as long as the hyperreactive state exists Evidence has already been deduced suggesting that such a mechanism may be operative in preventing the occurrence of Type 1 paralytic polio at least in some persons who have had a prior Type 2 infection

In such persons there is evidence of a heightened state of immunologic hyperreactivity to Type 1 virus This is shown in the second column of Figure 43 where you see a group of individuals who possess no antibody to Type 1 to begin with but do have antibody to Type 2 and they react to the Type 1 component of the vaccine more actively than do those who have no antibody to any of the 3 types They did not react as actively as those who have had a prior Type 1 infection This heightened state of immunologic hyperreactivity conceivably could be the critical factor in determining whether or not paralysis will ensue and will determine the degree of paralysis that might develop even though at the time of later exposure to Type 1 infection there is no demonstrable Type 1 antibody in the serum

The development of such cross immunity reaction seems to be due to the presence of small quantities of Type 1 antigenic groupings which are known to be present in naturally occurring Type 2 viruses It is remarkable that so small an amount of Type 1 cross relationship as seems to exist in certain strains of Type 2 virus is sufficient to bring about so significant a degree of alteration in the state of immunity to Type 1 paralysis A similar relationship between the Type 3 and the Type 1 virus is not prominent If the mechanism suggested is operative in the prevention of paralysis then it is of interest to know whether or not the hyperreactive state resulting from infection or from the injection of a killed virus vaccine is equally effective in the prevention of paralysis

The importance of this question arises from the fact that it is possible with a killed virus

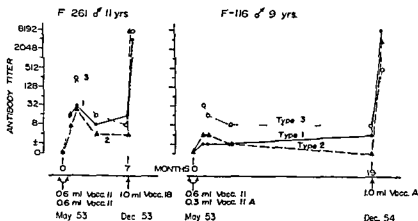


Fig 41 Immunologic hyperreactivity at 7 months and 19 months after primary vaccination

called on to assess the actual production and testing records of many commercial scale lots of vaccine produced by six manufacturers, it has had the exceptional opportunity of contrasting theories of the inactivation process with practical results. Contrary to published statements, the Committee has never taken a position that any one theoretical function or inactivation curve dealing with the loss of infectivity of polio virus under formaldehyde treatment was proved by the results of inactivation experience with commercial scale lots of vaccine. The theoretical considerations are of great importance, are controversial in some details, and require more investigation, but such investigations must be carried out with experimental lots much smaller than those which the Committee was called on to assess.

However, to the knowledge of the Committee, inactivation data of manufacturers throughout the world show beyond a doubt that the inactivation rate of polio virus as established by Salk is sufficiently approximate to a straight line on a semilog plot so that for practical purposes the time of inactivation of a pool in which all virus particles are accessible to formaldehyde should be predictable from the slope of the line. Recent workers have presented evidence that the inactivation process is more rapid during the first few hours after addition of formaldehyde than was realized before. Moreover, the exact shape of the formaldehyde inactivation curve of this and other viruses, especially after the first few days of inactivation, is still debated. It must be emphasized, however, that the differences of opinion do not influence the fact that completely satisfactory inactivation can be obtained by means of the present method of inactivation at 37°C with 1:4,000 formaldehyde.

One of the most important findings which has led to the development of a uniform process of vaccine preparation in the United States is the role of filtration or clarification of virus and vaccine fluids in facilitating the completion of the inactivation process. Very early in the analysis of production records it became evident that there was variation in the regularity with which the producers of poliomyelitis vaccine consistently achieved complete inactivation. Analysis by the Committee made it apparent that there were at least two major differences in the manufacturing processes being employed. One method involved the growth of polio virus for vaccine in cultures of minced kidney tissue and clarified the virus fluids by filtration through Seitz type pads, which, of course, are made of felted cellulose and asbestos fibers. A second method of production involved the growth of

TABLE 23 INFLUENCE OF TYPE OF FILTRATION ON COMPLETION OF FORMALDEHYDE INACTIVATION IN PRIMARY PROCESSING OF SINGLE STRAIN POOLS OF ALL 3 TYPES OF POLIO VIRUS

Material	Filtration Method	Total No. of Single Strain Pools	No. of Pools Incompletely Inactivated	Per cent of Pools Incompletely Inactivated
A Production from June 1, 1955 to Jan 1, 1956	Seitz Glass	94 174	2 16	< 1 9.2
B Production from Feb 1, 1956 to Feb 1, 1957	Seitz Glass	501 230	1 53	0.21 23.0
C Single Strain Pools from Trypsinized Cultures June 1, 1955 to Jan 1, 1956	Seitz Glass	6 172	0 16	0.0 9.3
D Single Strain Pools from Trypsinized Cultures Feb 1, 1956 to Feb 1, 1957	Seitz Glass	212 230	1 53	0.47 23.0

A and B include virus produced in Maitland type and trypsinized type cultures.
C and D include virus produced in trypsinized type cultures only.

virus for vaccine in trypsinized suspensions of monkey kidney cells and the clarification of virus fluids by filtration through fritted glass filters of graded porosities Fritted glass filters were being used because of experience suggesting that Seitz type filtration of virus grown in trypsinized cultures resulted in serious loss of virus titers The two methods yielded different results in the uniformity with which inactivation was achieved This is evident from the findings which I shall show in the following table (Table 23) In A and B one can see that *the success of inactivation of material filtered* through Seitz type filters in this production period was significantly greater than that with glass filtration namely 2 per cent of 94 versus 9 per cent of 174 single strain pools In a later production period an even greater difference is shown with a very successful or satisfactory effect of inactivation with Seitz filtered material

C and D in Table 23 show material uniformly processed from trypsinized cultures Here you see that the difference is still apparent and that the difference in favor of the Seitz filter must be attributed to the filtration and not to the method of preparation of the virus fluid The total experience vividly demonstrates the superiority of the method employing Seitz type filters

The Committee recommended that further experimental work be done and the results made available to the Committee may be summarized as follows

1 Seitz type filtration of the virus fluids during the formaldehyde inactivation process was much superior to filtration through fritted glass in yielding satisfactory vaccine In one laboratory 16 out of 46 glass filtered single strain lots of poliomyelitis virus failed to be completely inactivated satisfactorily by formaldehyde *These experimental lots of virus were* produced on a production scale during approximately the same period of time

2 Untreated polio virus grown in trypsinized culture can be satisfactorily filtered through Seitz type filter pads under proper conditions without significant loss of infectious titer This was not previously realized

3 Vaccine prepared from polio virus grown either in trypsinized or Maitland type cultures and clarified before and during the formaldehyde inactivation process by filtration through

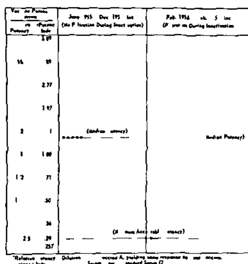


Fig 44 Type 1 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

Seitz type pads possesses satisfactory antigenic potency However after the introduction of the double Seitz filtration that is before and during the inactivation process the distribution of potency values of released vaccine lots was somewhat inferior to that obtained when single filtration through either glass or Seitz type filters was employed The depression of potency from this and probably other sources affects the Type 3 components especially The next 3 illustrations will show this effect

With the Type 1 (Fig 44) these are the potency indices in relation to a standard reference serum We see that the median potency of released vaccine lots during this period with no filtration during the inactivation process—filtration only prior—is higher than that of lots with *the added filtration during inactivation*

Type 2 shows a similar effect (Fig 45) Type 3 shows a much more pronounced effect which may be attributable not only to the second filtration but also possibly to other factors as well (Figs 46 A and B)

With the completion of experimental work indicating that pools of Seitz filtered polio virus were inactivated by formaldehyde with much greater regularity than those clarified by filtration through fritted glass and with the further establishment of the fact that antigenic potency

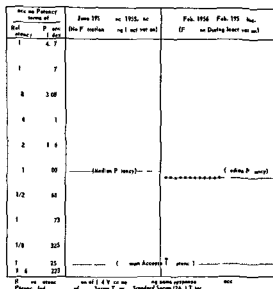


Fig 45 Type 2 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

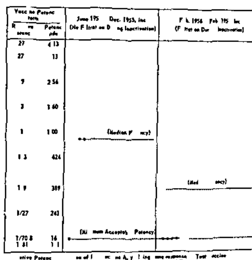


Fig 46A Type 3 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

was retained after Seitz type filtration the Committee recommended modification of the procedure for the manufacture of vaccine (Figs 47 and 48)

It seems evident from the results of these steps that the critical difference in the manufacturing processes was not the method used in producing virus as much as differing filtration practices This fact leads to the new and important observation that in practice the completion of inactivation of virus fluids depends on carefully controlled filtration procedures The extraordinarily high degree of homogeneity required for inactivation of the last few virus particles has not been previously recognized as a highly critical condition nor as a difficult achievement but it is probable that failure to achieve it resulted not only in serious difficulties in 1955 with some batches of polio vaccine but also with experimental batches of formalinized vaccine for Venezuelan equine encephalitis

In retrospect it appears that the advantages of preparing virus from monolayers of monkey kidney cells were unexpectedly outweighed by the more successful filterability of fluids from minced kidney cultures when Seitz pads were used Of course it is possible that still better methods may be devised for obtaining more efficient filtration of both types of fluids A better

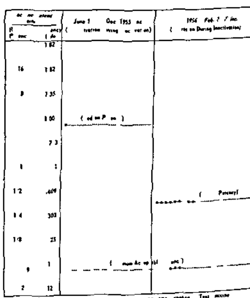


Fig 46B Type 3 Frequency distribution of potencies of released vaccine lots (all manufacturers by filtration procedures)

understanding and control of the intimate details of formaldehyde inactivation could in time enable a closer approach to the dual ideal of complete inactivation plus total retention of antigenicity Fundamental research in this field still seems to be desirable and the results could

ake possible great improvements in the production of many viral antigens

In relation to safety testing one important nding of the testing experience of vaccine was at the examination of vaccine for residual live rus is not comparable with the problem of easuring infective quantities in untreated ive virus suspensions Apart from statistical nsiderations of adequacy of sampling and of olume of material to be tested it is now clear at testing of vaccine in tissue cultures should accompanied by rigorous controls of the sen ivity of the tissue-culture system in each boratory and controls on the effect of the ccine fluid on the sensitivity of the system Vith proper control the tissue-culture system n be demonstrated to be superior in sensitivity the monkey safety test partly because of the uch greater volume of material tested or stable in tissue cultures Nevertheless the onkey test on a series of successive batches hen negative has always indicated a quality production associated with a safe product

However the inability of the monkey test to etect extremely low levels of infectivity in accine in comparison with large scale tissue ulture tests led to experiments designed to crease the susceptibility of monkeys in the st By a combination of intraspinal and intra uscular inoculation and with a variety of nhancing procedures two research laboratories btained many more infected monkeys with irus isolation than were obtained with simple ntracerebral monkey tests on the same lots of accine which epidemiologic evidence showed o be infectious These findings and other evi ence show that the monkey test could be rengthened by the use of multiple routes of noculation and of enhancing injections Such rocedures were incorporated into the current egulations

Although it is certain that the tissue-culture est at its best is more sensitive than any mon ey test now available the tissue-culture test nay fluctuate in sensitivity to a greater degree in ctual practice or may be less sensitive than the nonkey in the detection of protected particles f virus which escape inactivation Current nethods of production and testing since Oc ober 20 1955 have yielded with one possible xception more than 230 successive trivalent ots which were noninfectious in both the new monkey safety tests and in tissue-culture tests Most of these pools contained as much as 500 to 1 000 liters of vaccine each

The available evidence on current testing practices as well as the original ones supports the earlier expressed conclusion of the Commit tee that the significance of a negative test for infective virus on a given lot of vaccine is greatly enhanced if the particular lot is one of a series in which tests for infective virus are negative

One of the incidental findings in connection with the program of safety testing of vaccine in industry and one which has great interest in relation to production of any vaccines using viruses from animal or human sources is that many viruses were found to be latent in asymp tomatic monkeys used for production of kidney tissue cultures At least 28 distinct agents have now been identified which are either occasional contaminants of tissue cultures of monkey tissues or are present in the living animal and are acti vated in the process of cultivation of kidney cells These agents produce cytopathogenic effects in tissue cultures All of them are inacti vated by formaldehyde at least as readily as polio virus and therefore present a troublesome problem of identification rather than a threat to safety of the vaccine

Pre existing virus infections in monkeys used in the monkey safety testing have also been troublesome since the monkey safety test re quires histopathologic confirmation that asymp-tomatic test animals have not been infected by the vaccine Analysis of over 100 asymptomatic monkeys with inflammatory or glial lesions of the central nervous system indicates that a small proportion of test animals exhibit lesions of the viral type ranging from obviously old and healed lesions to extensive involvement in the subacute stage Five categories of lesion distri bution which were considered as atypical for poliomyelitis infection have been identified All were represented by instances in which lesions were so old as clearly to antedate the time of injection The need for reinforcement of the histopathologic criteria for poliomyelitis infec tion in doubtful asymptom tic cases has led to the recommendation that final diagnosis in such cases be based on attempts at virus recovery as well as histopathologic evidence This require ment subsequently led to the interesting finding that at least some of the atypical pathologic

TABLE 24 SURVEY OF TRIVALENT POOLS IN MONKEYS AND IN TISSUE CULTURE (5 INDUSTRIAL LABORATORIES)*

SURVEY PERIODS	NO POOLS	POOLS POSITIVE IN MONKEYS		POOLS POSITIVE IN TISSUE CULTURE					POOLS POSITIVE IN BOTH	POOLS POSITIVE IN BOTH TYPE
				ALL POOLS		VIRUS TYPES ISOLATED				
		NO	PER CENT	NO †	PER CENT	TYPE 1	TYPE 2	TYPE 3		
Prefield Trial and Field Trial Periods	53	5	9 4	2	3 8	1	0	1	2	1
From Field Trial Period to May 26 1955	146	1	0 7	17	12 0	6	6	11	0	0
From May 26 to Oct 20 1955	62	0	0 0	7‡	11 0	4	4	2	0	0
From Oct 20 1955 to April 1 1956	102§	0	0 0	0	0 0	0	0	0	0	0
From April 1 1956 to Oct 1 1957	128			1▼	0 78	0	1	0	0	0

For r sons beyond the control f the c mm ttee data for th s surv y are not a a lable from all 6 indu trial lab ratories The negativ pools which were deri d from the e perience of the 5 l borato s ho n for all 5 periods are correlated v ith a field e perience which appears to be entirely safe with the po ible exc ption of l vaccine lot from the second p iod

† Includes pools from which mo e th n l type w s i lated

‡ In ludes 4 pools f om a ingle lab ratory Remaining labor tories had a r te f 5 6 per cent p tive tr lent p ls in this peri d

§ Th e l ts actu lly er subject d t 209 parate n nkey fety te ts n a total of 4 850 m nk ys becau e of dupl cate tests by either the manuf ct r r or by the NIH T u -cultur te ts o these lots lso inv l d test sampl s often greatly in e ce of requ rement

▼ Th lot was co id r d as po ti alth ugh t c uld not be e t bl hed co lu ly that the v s olat d v as der ved from th ac in lot rather than f om con t min tion f th t u cultu u ed n the t t

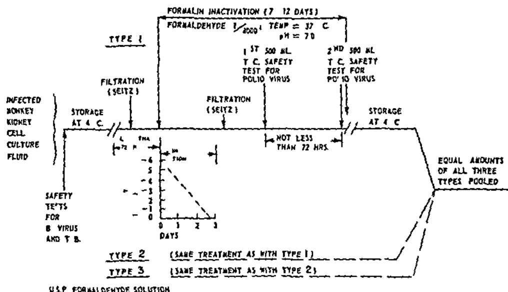


Fig 4 Single strain pool

pictures observed in test animals were associated with the recovery of the B virus of Sabin and Wright The abortive nature of the infection of the central nervous system in such asymptomatic animals despite the treatment with cortisone which was administered at the time of inoculation suggests that this virus may be activated by the trauma of the injection into the nervous system

In the monkey potency test the antibody response elicited in simians by injection of the undiluted vaccine being tested is compared with that previously elicited by 3 different dilutions of standard vaccine In preparing the antisera against the standard trivalent vaccine large groups of monkeys were immunized with undiluted 1 4 and 1 16 dilutions of a reference vaccine material from the 3 pools of antiserum were subsequently employed for the comparison with the antiserum produced with each batch of undiluted vaccine being tested This test served an invaluable function during the first few years of vaccine production Its results have correlated reasonably well with efficacy of the vaccine

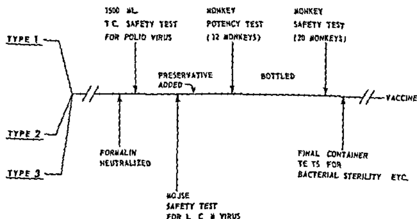


Fig 48 Trivalent

in human populations as far as limited data show Its deficiencies have been recognized wherever it has been used in large scale production A test using small animals and capable of greater precision through the use of larger numbers of animals offers p actical advantages and many European laboratories soon adopted the guinea pig test developed by Gard in Sweden In this test an antigenic extinction titer is obtained by vaccinating groups of animals with successive dilutions of va cine The lowest concentration producing an antibody response represents the level of antigenic extinction and is a measure of the potency of the vaccine A similar test in chicks has been reported and is being developed in an extensive co-operative study by the United States manufacturers and the United States Public Health Service Early results appear promising

With such tests in small mammals or chicks a statistically adequate number of animals can be used with considerable economy of effort animals and antigen The use of a live virus standard is also possible and affords an ideal target for improvement of potency since the tar get is the ultimate level achievable by a live nonmultiplying viral antigen Finally in a single completely controlled test in a single batch of animals the standard antigen can easily be compared with a series of test samples whether these are inactivated by a single method for routine production or are part of an experi mental series designed to study different meth ods of inactivation and their effects on potency

It is now clear that considerable variation in the potency of different batches of vaccine still exists The improvement of the means for con trolling potency of the vaccine may seem to be a technical detail but on this detail rests the most important remaining problem of vaccine production namely improving the potency and the uniformity of potency of the vaccine The development of a convenient and accurate method of potency testing will be an important aid in achieving this objective

After the unfortunate incident in the spring of 1955 in which the Mahoney Type I strain was clearly shown to be virulent in human beings as it is in monkeys a considerable effort was made to find a less virulent strain with equal or better immunizing capacity as well as with the necessary characteristics of stability during the manufacturing process The problem has been more difficult than was anticipated Although strains of relatively low virulence are available no proposed substitute strain which was tried has shown properties during large scale manufacture of vaccine which indi cate a clear superiority over the Mahoney strain in antigenic potency in human beings Obvi ously the search must continue to find a Type I substitute as superior in potency to the Mahoney strain as is the Type 2 component in the vaccine which approaches the antigenic potency of un inactivated virus The vaccine could also be improved by means of a more stable Type 3 component and there is little doubt that an inactivated vaccine composed of 3 strains equal to the Type 2 component would be greatly supe rior to any vaccine now being produced

Looking back at the diffi ulties encountered in the large scale manufacture of formalinized polio vaccine one can see that much of lasting value has been learned in the aftermath Path ways to improvement of the vaccine and of its control have been marked out Most of all the facts and real achievements which were recog nized at the last Conferen e and after the Field Trial of 1954 have stood the test of time of temporary confusion and of controversy The result is a widely used and widely accepted prep aration of demonstrated safety which is of high protective value against paralytic poliomye litis

The vaccine discussed here is the one which has been most extensively tested for its pre ventive power against paralytic poliomyelitis

BIBLIOGRAPHY

Bodian D The enhancement of susceptibility of monkeys to polioviruses of high and of low virulence Am J Hyg 64 92 1956

Cartwright T E Ritchie A E and Lauffer M A The reaction of tobacco mosaic virus with formaldehyde III Kinetics of the loss of infectivity Virology 2 689 1956

Cornfield J Halperin M and Moore F Some statistical aspects of safety testing the Salk poliomyelitis vaccine Pub Health Rep 71 1045 1956

Eklund C M Bell E J and Hadlow W J Detection of live virus in monkeys in certain lots of poliomyelitis vaccine by inoculation of monkeys Am J Hyg 64 85 1956

Francis T Jr Korns R F Voight R B Boisen M Hamphill F M Napier J A and Tolchinsky E An evaluation of the 1954 poliomyelitis vaccine trials summary report Am J Pub Health 45 1 1955

Gard S Chemical inactivation of viruses *in* Ciba Foundation Symposium on the Nature of Viruses pp 123 141 London Churchill 1956

Gard S Wesslen T Fagraeus A Svedmyr A and Olin G The use of guinea pigs in tests for immunogenic capacity of poliomyelitis virus preparations Arch ges Virusfor 6 401 1956

Hull R N Minner J R and Smith J W New viral agents recovered from tissue cultures of monkey kidney cells I Origin and properties of cytopathogenic agents S V$_1$ S V S V$_4$ S V S V$_6$ S V$_{11}$ S V$_1$ S V$_{15}$ Am J Hyg 63 204 1956

Langmuir A D Nathan on N and Hall W J Surveillance of poliomyelitis in the United States in 1955 Am J Pub Health 46 75 1956

Sabin A B and Wright A M Acute ascending myelitis following a monkey bite with the isolation of a virus capable of reproducing the disease J Exper Med 59 115 1934

Salk J E Considerations in the preparation and use of poliomyelitis virus vaccine J A M A 158 1239 1955

——— Poliomyelitis vaccination in the fall of 1956 Am J Pub Health 47 1 1957

——— Vaccination against paralytic poliomyelitis performance and prospects Am J Pub Health 45 575 1955

Smith D G Mamay R K Marshall R G and Wagner J C Venezuelan equine encephalomyelitis laboratory aspects of 14 human cases following vaccination and attempts to isolate the virus from the vaccine Am J Hyg 63 150 1956

Summary of Technical Report on Poliomyelitis Vaccine Pub Health Rep 70 738 1955 (Full report on file at National Institutes of Health)

Syverton J T Brunner K T Tobin J O H and Cohen M M Recovery of viable virus from poliomyelitis vaccine by use of monkeys pretreated with cortisone and x radiation M J Hyg 64 74 1956

Technical Committee on Poliomyelitis Vaccine Interim report J A M A 159 1444 1955

Technical Committee on Poliomyelitis Vaccine and Subcommittee on Improvement and Simplification of the Manufacturing Process of Poliomyelitis Vaccine The role of filtration in the commercial production of poliomyelitis vaccine (In Preparation)

Technical Committee on Poliomyelitis Vaccine and Subcommittee on the Monkey Safety Test The monkey safety test for poliomyelitis vaccine Am J Hyg 64 104 1956

Timm E A McLean I W Jr Kupsky C H and Hook A E The nature of the formalin inactivation of poliomyelitis virus J *Immunol* 77 444 1956

Timm E A Pope E Z McLean I W Jr Chick potency testing of poliomyelitis vaccine basic studies of response (In Press)

U S Dept of Health Education and Welfare Public Health Service National Institutes of Health Minimum Requirements—Poliomyelitis Vaccine Bethesda Md 1954

U S Public Health Service Regulations—Biologic Products—Additional Standards Poliomyelitis Vaccine 21 Fed Reg p 4972 1956

Results Obtained by Means of Vaccine Composed of Inactivated Viruses

DR ALEXANDER D LANGMUIR

In April 1955 when Dr Francis reported on the success of the field trials of inactivated polio virus vaccine a comprehensive mass immunization program was initiated in the United States and has continued to the present time The objective is to achieve universal immunization of the 109 000 000 persons under 40 years of age in our population Many problems have been encountered Some were anticipated and planned for others were unforeseen and had to be met on an emergency basis Up to April 1957 approximately 145 000 000 doses of vaccine were distributed A little over half of the goal has been achieved for our population under 20 years of age Immunization of the adults from 20 to 40 has only barely begun and will not be pushed until more adequate supplies of vaccine become available to us Other nations notably Denmark have made far greater progress and their experience should be studied with care But it is also believed that our experience is of some interest from the sheer magnitude of its scope and from the extent of the evaluation which has been attempted The present paper will summarize the field epidemiologic aspects during 1955 and 1956 with particular emphasis on the evaluation of the safety and the effectiveness of the vaccine and of its use under epidemic situations

The information to be presented is a summary of the voluminous reports that have been collected in the Poliomyelitis Surveillance Program This is a major national co-operative effort which includes all states more than 40 virus laboratories many other co-operating organizations and more than 40 epidemic intelligence service officers who have been detailed for full time field duty or at least alerted for first priority duty in any field activity relating to poliomyelitis This program is continuing at full scale During its early stages primary concern was directed to investigation of the Cutter incident Later emphasis was placed on the evaluation of the effectiveness of the vaccine but at all times a constant surveillance has been maintained on safety of the vaccine actually distributed and used

SAFETY OF THE VACCINE

There were 3 distinguishing epidemiologic characteristics of the Cutter cases First excessive frequency of cases in association with the use of certain particular lots of vaccine this frequency being independent of the prevalence of naturally occurring poliomyelitis in the area. Second a concentration in the interval between inoculation and onset within the period of 4 to 11 days for the vaccinated cases and 8 to 27 days for the contact cases—this 8 to 22 days being essentially a double incubation period Third correlation between the site of the inoculation and the site of the first paralysis Now these 3 crucial features were essentially similar to those described for experimental inoculation poliomyelitis in cynomolgus monkeys by Bodian

The continuing surveillance for the safety of poliomyelitis vaccine in the United States has been based on constant earch for these distinguishing characteristics It was anticipated that as vaccine became more widely used purely coincidental cases of polio among vaccinated persons would be reported with increasing frequency This of course has occurred

In the calendar year of 1956 reports were received on 279 cases of paralytic polio which occurred in individuals who had received polio vaccine within 30 days prior to onset This total does not include a group of more than 300 similar cases from the Chicago epidemic which will be discussed later and separately

These 279 cases occurred in the large population that had received over 70 000 000 doses of vaccine during the period thus giving a ratio of about 1 paralytic case to every 300 000 inocu

lations Now the characteristics of these cases are of particular interest They were not associated in excessive frequency with any specific lots of vaccine but on the contrary tended to concentrate in areas where poliomyelitis was prevalent There was no concentration of the intervals between inoculation and onset in the 4 to 11-day period Paralysis occurred more than twice as frequently in the legs as in the arms which was a common site for inoculation While there are obvious limits to this type of epidemiologic evaluation and while it must be recognized that the concept of absolute vaccine safety is not scientifically tenable it is possible to conclude that the frequency of any hypothetical untoward effects resulting in the occurrence of poliomyelitis was less than 1 per million inoculations Thus it is clear that the more than 100 000 000 doses of vaccine distributed in the United States since the Cutter incident have been safe

NONSPECIFIC REACTIONS FOLLOWING INOCULATION

As part of the surveillance of vaccine safety data have been collected on vaccine reactions Among those reactions considered to present a possible hazard were encephalitis or other neurologic illness such as may occur following use of smallpox or other vaccines allergic reactions to the traces of penicillin and other foreign proteins in the vaccine nephritis or other renal disease attributable to residual monkey kidney proteins in the vaccine and finally sensitization of Rh negative persons to antigen potentially present in the vaccine

To evaluate these problems the Poliomyelitis Surveillance Program has collected reports of a number of nonpolio illnesses occurring shortly after vaccination On the basis of reports that have been received it is evident that vaccine reactions of all types both mild and severe have been rare It is apparent that poliomyelitis vaccine is associated with at least as low a frequency of reactions as any other immunizing agent in common use today

VACCINE EFFECTIVENESS

During the nationwide campaign for mass vaccination it has not been possible to conduct carefully controlled studies to evaluate effectiveness of the vaccines in actual use Instead evaluation has depended on more qualitative types of studies and on orderly epidemiologic inference based on careful observation and analysis The consistency with which many different independent studies have indicated a substantial effectiveness against paralytic cases is impressive

The following types of study will be summarized the total incidence in the nation analysis of the age distribution of cases the occurrence of paralysis in relation to past vaccination studies in one particular state California and then careful studies of epidemic situations that we have faced

The annual incidence rates of total reported cases—this includes both paralytic and nonparalytic cases which is customary in the United States—from 1910 to 1956 are shown in Figure 49 The weekly incidence for the 11 years from 1946 to 1956 is shown in Figure 50 A total of 15 000 cases were reported in 1956 as compared with 28 000 cases in 1955 It is necessary to go back to 1947 to find a year with lower total reported incidence While the widespread use of vaccine in 1956 undoubtedly has influenced this incidence the experience of several more years will be necessary before the broad effect of mass vaccination can be judged adequately In our own group we tentatively set 1947 as the low mark beyond which we must go before we can draw confident conclusions from these types of crude epidemiologic data

During 1955 33 states co operated with the Polio Surveillance Program in reporting the age the paralytic status and the vaccination history of reported cases of polio During 1956 these 33 states and 12 others joined in this co-operative effort During the spring and the summer of 1955 the utilization of polio vaccine was limited to the first and second grade school children because of the short supply As a result a high proportion of 7 and 8 year-old children had received at least 1 dose of vaccine before the peak of the 1955 season The age specific attack rates for reported paralytic cases were significantly lower than in the younger age groups or in the older children through age 14 as is shown in Figure 51

Note particularly the dotted line which shows the 1955 incidence there is a notch or a defect in the curve out of character with all previous years experience which relates to the 2 years of age that had received polio vaccine during the

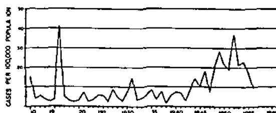


Fig 49 Annual poliomyelitis incidence rate in the U S 1910 1956 (U S Public Health Service)

NFIP supported clinics in 1955 These lower rates in the 7 and 8 year-old children were interpreted to reflect a higher degree of immunity in these groups resulting from the vaccination

Now in 1956 vaccine became increasingly available During the winter and the early spring priority was given in most areas to the 5 to 9 year age group but by April the priority was extended to the age span from 0 to 15 years of age and to pregnant women By late summer vaccine became available for all under 20 years of age Specific figures of the proportion of the population immunized by individual age groups are not available for the nation as a whole However it can be conservatively estimated that by September 1—essentially the peak of our polio season—approximately 50 per cent of the whole group from 1 to 19 years of age had received one or more doses of vaccine

Therefore the age specific attack rates for 1956 are of particular interest You will note that for 1956 the solid curve is shifted sharply to the left (Fig 51) The peak incidence occurred in the 1 year age group instead of in the 2 to 5 year group as in 1955 The rate in 1956 fell sharply and steadily from the high in 1 year-olds to a low in 9 year-olds after which it rose slightly and remained level through age 30 The low rates in 1956 in the 8 and 9 year-olds correspond to the low rates in the 7 and 8 year olds of 1955 Since these age groups represent the same children who received first priority in vaccination in 1955 the continuing low rates point to a duration of effectiveness of vaccine for at least 1 year

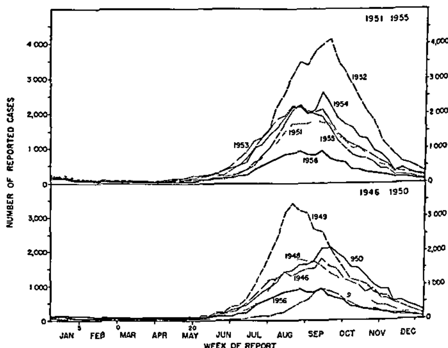


Fig 50 Incidence of poliomyelitis in 1956 as compared with years 1946-1955 (U S Public Health Service)

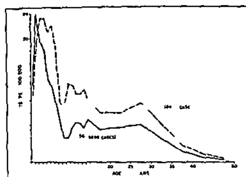


Fig 51 The age distribution of paralytic poliomyelitis in 1955 and 1956 (data from 33 states July-October 1955 and calendar year 1956) (U S Public Health Service)

HISTORY OF PARALYSIS IN RELATIONSHIP TO HISTORY OF VACCINATION

During 1956 52 per cent of the total cases of poliomyelitis were classified as paralytic according to the records submitted in the Age Distribution Study This over all figure is comparable with similar types of data reported in 1955 and again in 1952 When cases reported in 1956 in the under 20 year age group are separated by vaccination history it is found that only 34 per cent of vaccinated cases were paralytic whereas 60 per cent of unvaccinated cases were reported as paralytic This difference indicates that the vaccination prevented some paralytic cases

In a study of only the paralytic cases occurring during the midsummer season in the under 20 year group 21 per cent had a history of vaccination This figure may be compared with the previously mentioned estimate that approximately 50 per cent of this age group had received 1 or more doses of vaccine by midseason This difference points to a vaccine effectiveness of the order of 75 per cent against paralytic polio

In the Age Distribution Analysis the cooperating states were not asked to report on the number of doses of vaccin that each vaccinated case had received A special study of hospitalized cases was performed independently by the National Foundation for Infantile Paralysis which did collect data on numbers of doses of vaccine received Reports were received on 3 198

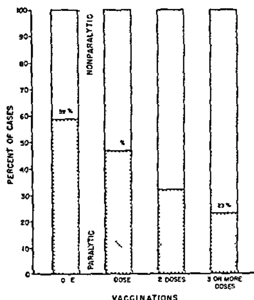


Fig 52 Frequency of paralysis in polio myelitis patients by vaccination history August 15 September 30 1956 (data from a hospital survey by the NFIP) (U S Public Health Service)

acute admissions for polio in 408 hospitals in 48 states The results are shown in Figure 52 When the cases are grouped according to the number of vaccine doses received prior to onset the frequency of paralysis declines progressively from 59 per cent among unvaccinated cases—which agrees so closely with our figure of 60 per cent—to 23 per cent among those who had received 3 doses This study not only supports the effectiveness of the vaccine in reducing paralysis but also emphasizes the importance of the full course of 3 doses

STUDIES IN PARTICULAR STATES

During 1956 special studies were more difficult to conduct because of the lack of precise data on utilization of vaccine in specific age and geographic groups In the state of California however a very intensive effort was made to follow the vaccine distribution month by month and thus the effectiveness could be measured with great care This study recently released for our general information maintains an effective ness of well above 75 per cent for those who had received more than 1 dose

EPIDEMIC STUDIES

In 1955 a severe epidemic of polio struck in Boston and extended over the entire eastern half of the state of Massachusetts An extensive study of this epidemic was made by Pope Feemster and co workers and others Special attention was directed to the effectiveness of vaccine in first and second grade school children A school census in that state provided accurate data on populations exposed to infection in various age groups

The attack rates were high not only in the crowded areas of the city of Boston where the epidemic appeared to originate but they also remained high in the suburban areas and in the smaller communities around Boston There was no apparent concentration of cases in particular socioeconomic racial or geographic groups Such almost uniform distribution of disease over a wide area is characteristic of most large scale epidemics of polio in the United States

This study in Boston permitted a large scale test of the effectiveness of 1 dose of vaccine under severe epidemic conditions The results showed an overall effectiveness of 53 per cent against all types of disease including nonparalytic cases and an effectiveness against paralytic cases of 60 per cent Effectiveness was found to be equally good for 6- and 7 year-old children as well as for 8 and 9 year-old children thus helping to resolve one of the questions raised in the findings of the Francis Report Also—and I believe of great importance—the effectiveness of the vaccine was found to be greater *against the more severe forms of the disease* It was 69 per cent effective against severe and fatal polio

In Chicago an epidemic of polio began in

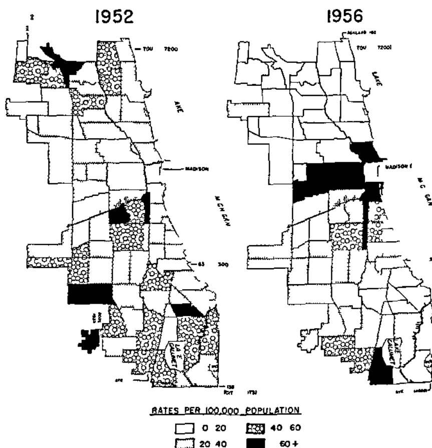


Fig 53 Poliomyelitis incidence rates in the community areas of Chicago (U S Public Health Service)

June 1956 The incidence increased rapidly to a peak in the first week of August and then as rapidly declined A total of 1 106 cases was reported During the last previous polio epidemic in that city which occurred in 1952 a similar number of cases was reported namely 1 203

Despite the similarity in incidence that is in total cases during these 2 epidemics there was a striking contrast in other epidemiologic characteristics In 1952 the cases were widely distributed throughout all areas of the city as is shown in Figure 53 In only a few of the 73 community areas was the rate lower than 20 per 100 000 The disease appeared to attack negroes and whites in all socioeconomic groups in the essentially uniform pattern that is characteristic of large poliomyelitis epidemics Note on the left hand side of the illustration that in the 1952 distribution of cases throughout the northern part of the city which is a middle and upper-class area there was heavy polio There were scattered areas here with high and low rates and again high rates in parts of the southern part of the city of middle-class population In 1956 this northern area was almost wholly free of disease large parts of the south were free and there was great concentration in the middle range and down in the south rn tip which characteristically are now occupied by negroes

In contrast the cases during 1956 were sharply concentrated in the community areas known to have the lowest socioeconomic status and the highest proportion of negroes In these areas little opportunity has been provided for poliomyelitis vaccine particularly for preschool children *Large areas in the north and the south* sides of the city were spared These areas house the middle and the upper socioeconomic groups These groups had greater opportunity to receive polio vaccine through their private physicians

Other changes in the epidemiologic pattern occurred in 1956 There was a marked shift in incidence to younger age groups 39 per cent of the white cases and 69 per cent of the nonwhite cases being under 5 years of age in 1956 as compared with 26 and 46 per cent respectively in 1952 Thus the epidemic in Chicago in 1956 presented unique features In spite of wide spread seeding of the virus throughout the city epidemic rates developed only in a few areas and in the specific age and racial groups containing small numbers of vaccinated persons

Many authorities have expressed fear that the administration of polio vaccine during an epidemic might result in localization of paralysis due to the so-called provoking effect known to occur following other types of inoculations Hence in 1955 in Massachusetts in Wisconsin *and other areas vaccination was stopped as soon* as the existence of epidemics was realized Therefore no opportunity was presented to test whether or not this fear of the provoking effect was real or to measure the quantitative importance of it

In October 1955 an epidemic of polio broke out among naval dependents in Pearl Harbor Hawaii In the face of a rising incidence of the disease vaccination was given to over 20 000 individuals comprising 80 per cent of the population at the Naval Base Careful studies by Poos and Nathanson failed to demonstrate evidence of a provoking effect

This one study cast doubt on the existence or at least on the importance of this hypothetical hazard In 1956 when epidemic polio broke out in Chicago mass immunization was conducted during the period of the peak incidence Particular attention was directed to the areas in the city experiencing the highest rates During a 6 week period from July 20 to September 1 1 750 000 doses of vaccine were administered Special studies were conducted by a team from the Communicable Disease Center Over 1 100 cases of polio were reported during this year (1956) Of these about 300 had a history of recent vaccination of which 75 per cent were paralytic Among these first paralysis developed in the legs twice as often as in the arms In those with arm paralysis the sites of inoculation were approximately equally distributed to the paralyzed and the unparalyzed sides There was no evidence of concentration in an interval of 4 to 11 days Thus there was no evidence found of a provoking effect It is concluded that this hypothetical hazard is not of sufficient consequence to inhibit mass vaccination in epidemic situations

The usefulness of mass vaccination in the face of a rising incidence or an epidemic prevalence of polio requires further evaluation In the Hawaiian epidemic the incidence dropped sharply about 4 weeks after the mass vaccination began Later cases were limited largely to

new arrivals While this experience is suggestive it cannot be considered as conclusive evidence that mass vaccination was responsible for the termination of the epidemic

In Chicago the mass vaccination began intensively in the last week in July The peak incidence occurred during the week ending August 3 Such an early peak is most unusual in Chicago the first of September being the date of expected maximum incidence However the Chicago epidemic began earlier in 1956 than in other epidemic years and the peak occurred sooner than could be expected to have resulted from the mass vaccination program itself Preliminary studies of the epidemic curve failed to show an accelerated decline or other influence that can be ascribed with any assurance to the immunization program It is probable that the campaign began too late to exert a material influence on the natural course of the epidemic

SUMMARY

In the United States experience with vaccine since the Cutter incident shows that the vaccine has been safe and almost free of severe untoward reactions of a nonspecific character

The effectiveness of the vaccine has been maintained at the level of approximately 75 per cent or better Most observations have been based on populations where the majority of persons have received only 1 or 2 doses The effectiveness of a full course of 3 doses of potent vaccine properly spaced has not yet been measured Such indications as are available point to an effectiveness greater than 75 per cent The duration of immunity has not yet been determined Several more years of study both in the field and in the laboratory will be necessary to determine how long immunity lasts and how frequently booster inoculations should be repeated

Experience with mass vaccination first in Hawaii and then in Chicago has allayed the fear that such a procedure might provoke the localization of paralysis at the site of inoculation This fear no longer justifies avoiding mass vaccination in areas with high prevalence While mass vaccination in all future epidemic situations will probably be administratively unavoidable it is doubtful that a large enough proportion of the susceptibles in the population can be immunized in time to influence the course of the epidemic This I would restrict to the sharp epidemics of the temperate zones rather than in areas experiencing a longer and more protracted outbreak Very likely vaccination would show a measurable effect in the latter epidemic situation Wise health officials will endeavor to achieve a high level of immunization prior to the time when poliomyelitis epidemics may occur and thus forestall them

CONCLUSION

Our experience with inactivated polio virus vaccine has been encouraging With continuing progress in achieving increasing levels of immunization in all segments of our population under *40* years of age a steady reduction *in the frequency* of paralytic cases can be confidently anticipated

BIBLIOGRAPHY

Bodian D Viremia in experimental poliomyelitis Am J Hyg 60 358 1954

Feemster R and Pattison E Personal communication

Francis T Jr *et al* Evaluation of 1954 poliomyelitis vaccine trials summary report Am J Pub Health 45 1 1955

Langmuir A D Nathanson N and Hall W J Surveillance of poliomyelitis in the United States in 1955 Am J Pub Health 46 *75 1956*

Philbrook F R and Ingalls T H Poliomyelitis vaccination in southeast Massachusetts in 1955 influence of a single dose of Salk vaccine New England J Med 255 385 1956

Poliomyelitis Surveillance Unit Surveillance of poliomyelitis in the United States in 1956 Pub Health Rep 72 391 1957

Poos R S and Nathanson N *Use of polio*myelitis vaccine under epidemic conditions *J A M A 162 85 1956*

Pope A S Feemster R Rosengard D E Hopkins F R B Vanadzin B and Pattison E Evaluation of poliomyelitis vaccination in Massachusetts New England J Med 254 110 1956

U S Public Health Service Report on Poliomyelitis Vaccine Produced by Cutter Laboratories (Official Release) August 25 *1955*

——— Technical Report on Salk Poliomyelitis Vaccine (Official Release) *June 10 1955*

World Health Organization Poliomyelitis Vaccination A Preliminary Review (WHO Technical Report Series No 101) New York *Columbia 1956*

DISCUSSION

DR VON MAGNUS The short time which has elapsed since vaccination started does not allow final conclusions as to duration of immunity Dr Salk has a series of observations which give him confidence on this point Much can be said about the problem of duration of immunity Even live virus vaccines presently used are not regarded as giving a lifelong immunity Thus for in tance an international smallpox vaccina tion certificate is valid for only 3 years

The complications to the vac ination in the United States have been few—fewer than could be reasonably expected Observations in our country were approximately the same as those reported by Dr Langmuir In our country ? 500 000 individuals have been vaccinated in tradermally 2 or 3 times by now Various observations on this vaccination program are pre sented in the Scientific Exhibits and I will not comment on it here

As far as safety is concerned I will remind you of a sentence by Dr John R Paul from his closing remarks at the Conference in 1954 Re ferring to the future use of a killed polio vac cine he stated Acci lents are bound to hap pen" As we all know an accident did happen. The impression on people in all parts of the world was tremendous But perhaps it can be said and personally I feel it very deeply in deed that this unfortunate accident which hap pened early at the very beginning of the mass vaccination program in the United Sta es has prevented a number of pos ibly even more se vere accidents in connection with vaccines pre pared in other parts of the world With the careful controlled procedures now in use the produ t can be said to be safe as stated by Drs Bodian an l Langmuir

The need for polio vaccine is great in many countries and in most places there will not be enough vaccine for the first few years Even if the United States and maybe other countries at some point in the future may have a real surplus of vaccine economic considerations and cur rency problems most certainly will be a barrier for the distribution of this surplus to a number of other countries

Since we in Denmark have used the intra dermal route for inoculation of polio vaccine and since less volume of vaccine is used per in dividual by this mode of injection we have been asked by health authorities in a number of other countries whether we would advise them to use the intradermal route in their own country or not On an experimental scale Dr Salk has been able to induce antibody formation to all 3 types of virus in 100 per cent of the vaccinated whether the vaccine was given intradermally subcutaneously or intramuscularly Others have made similar observations on an experimental scale

However vaccines prepared on a larger scale and used so far in mass vaccination programs are less antigenic and to my knowledge do not induce antibodies in 100 per cent of those vac cinated The data available to us at present on vaccination with su h va cines in animals and in humans indicate that 1 ml of vaccine given subcutaneously yields a better protection for an individual than 1/5 or 1 4 of 1 ml given intra dermally However our data also support the obs rvation made by Dr Salk several years ago that one obtains a better utilization of a given amount of vaccine and more people are pro te ted if the intradermal route is used

DR HILL In Great Britain we have traveled a slightly different road and the contrast is per haps of interest In th British vaccine the Brunenders Type I strain replaced the more virulent Mahoney Early la year it was admin istered in 2 doses at an interval of 3 to 4 weeks to nearly 150,000 children aged 1 to 9 years They were chosen randomly according to their month of birth from nearly 2 000 000 children whose parents registered them for vaccination but for all of whom supplies were not available Subsequent observations showed an incidence of paralytic disease in the vaccinated children only about one fifth of the incidence in the unvacci nated Thus in 75 000 vaccinated children aged 5 to 9 years 1 case of paralytic poliomyelitis oc curred at the attack rate of the unvaccinated children there would have been 6 In 75 000

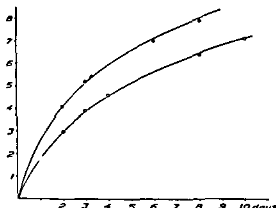


Fig 54 Inactivation of polio virus by formaldehyde Abscissa time in days Ordinate inactivation in log units Curves = equation (Wesslen Lycke Gard and Olin Arch Virusforsch 7 184)

vaccinated younger children aged 1 to 5 there were 3 paralytic cases at the attack rate of the unvaccinated children there would have been 15 These differences are statistically significant to use the magic phrase but the numbers are obviously too few to define accurately the level of protection induced At its face value it is about 80 per cent much the same as was noted in the original American trial And as in that trial the incidence of nonparalytic poliomyelitis appeared to be unchanged

In short the assessment of this British vaccine in the 1956 poliomyelitis season gave two important answers It showed that the milder Brunenders strain conferred protection against Type I infections it showed that protection could be given to the preschool child a point as Dr Langmuir points out not determined in the original American trial

As stressed by Dr Salk two unresolved problems lie in the duration of immunity and in ways and means of increasing the protection given to nearer 100 per cent On the duration of immunity we in Britain cannot add evidence on a strictly controlled basis In fulfillment of a promise we have been busily vaccinating our previous control children that is the registered but unvaccinated children Therefore we can make no further controlled observations Like Dr Langmuir we shall have to fall back upon the inferior evidence of secular trends The serious difficulties of interpretation were well illustrated in one of his figures

Clearly too it is impossible to use controlled field trials to answer the many questions raised by Dr Salk—the amount of antigen to use the intervals between injections Practice inevitably will have to rest upon evidence of the antigenic stimulus produced However there is one question of fundamental importance — whether 3 doses are substantially more effective than 2 The proportional and scanty figures from the questionnaire mailed to hospitals given by Dr Langmuir clearly cannot carry complete conviction as he himself was most careful to point out At present I know of no other satisfactory statistical evidence and in particular I doubt the figures collected during the Chicago epidemic

But to reach a sound conclusion on that point would need a field trial on a vast scale Suppose the 2 injections reduce a normal attack rate of 20 per 100 000 by three quarters that is to 5 per 100 000 Suppose that 3 injections might further reduce it to 2 per 100 000 that is by 90 per cent, all told To substantiate that change from 5 to 2 would call for a strictly controlled trial involving well over a million children I am not at all sure that such a trial can be made I myself have no doubt whatever of its importance

Dr Gard I cannot agree with Dr Bodian on the question of the kinetics of virus inactivation nor on the advisability of repeated filtration in the course of the process At the previous conference in Rome I reported the observations of a Swedish team that the course of the reaction deviated significantly and systematically from that of a first order These studies have been continued and extended mainly by Lycke in our laboratories

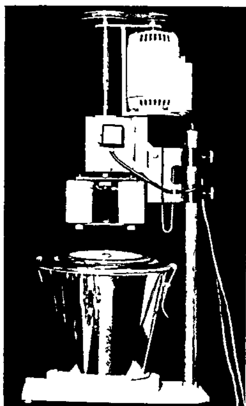
We have found that the reaction is adequately described by the formula

$$\log y - \log y = a \log (1 + bt)$$

(y = initial activity y = activity at time t)

I direct your attention to the fact that 2 constants are contained in the formula the constants *a* and *b* We will have an opportunity to mention those later Figure 54 shows the fit of observed data to the theoretical formula — the curves representing the formula

As the formula has been found to apply to a number of viruses tested (not only polio viruses



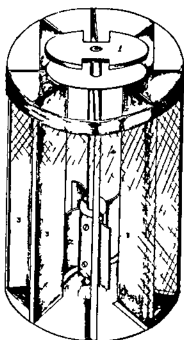


Fig 55 (*Left*) Homogenizer used in inactivation experiments (*Right*) Interior of homogenizer

of all 3 types but also Theiler s virus bacteriophage influenza virus foot and mouth disease virus and tobacco mosaic virus) it appeared to be a formula of universal applicability and therefore not to be affected by more or less incidental variations like aggregation or precipitation in the medium This has been borne out in systematic studies in our l boratory Thus we have found that filtration chemical purification treatment by enzymes and mechanical homogenization have had no eff ct whatsoever on the course of the reaction I can show one example

Lycke has designed a very powerful homogenizer It is a stainless steel container which is hermetically sealed (Fig 55) Above it you see an electromagnet that drives the movable parts The interior of this mixer is also shown The propeller spins at a rate of 600 rpm passing at a very short distance from a number of stationary lamellas In addition to the mixing effect of this device very high velocity gradients are produced each time the propeller blades pass the

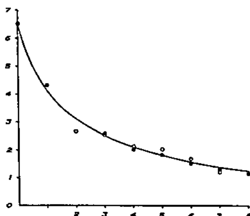


Fig 56 Inactivation by formaldehyde with (o) and without (•) continuous homogenization

lamellas actually 4 000 cm /sek /cm at a frequency of 320 times per second

Figure 56 shows an experiment where two

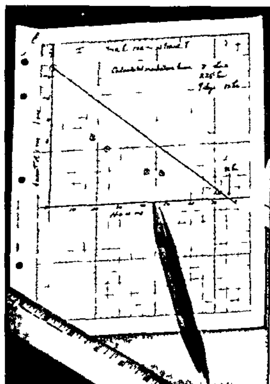


Fig 57 Inactivation curve

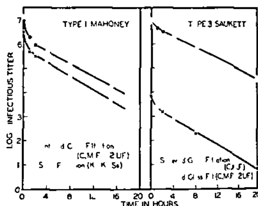


Fig 58 Effect of filtration on inactivation by formaldehyde (Timm McLean Kupsky and Hook *J Immunol* 77 444)

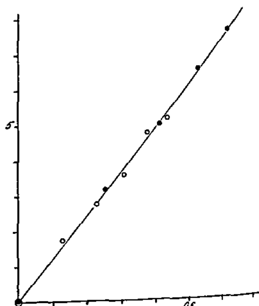


Fig 59 Inactivation by formaldehyde Author s observations on 21 vaccine batches = o Date of Haas et al from 60 batches = • Abscissa log (1 + bt) Ordinate mean inactivation in log units Parameters $a = 12\ 5$ $b = 0\ 339$ and $0\ 779$ respectively

identical lots of virus were treated with formaldehyde one under continuous homogenization day and night in the device previously shown the other without any stirring whatsoever As you can see both curves take exactly the same course

Dr Bodian referred to the world wide experience from commercial manufacturers I have to confine my remarks to what has been made public First I would like to present an illustration (Fig 57) showing how to draw a straight line through observed points Figure 58 likewise shows curved lines Incidentally it also confirms our experience that filtration has no effect on the shape of the curve it only retains a certain amount of virus As you can see the titers were greatly diminished after filtration

Finally only recently data from a large number of routine batches from the Behringwerke in Germany were published We have fitted the German results to our theoretical formula (Fig 59) Now this is a special representation The 2 constants *a* and *b* were calculated and log inactivation was plotted against the expression log $(1 + bt)$ The result should be a straight line if the data fit the formula As you can see the black points the German data fit extremely well and they also agree fairly well with *our* results the open circles On the basis of this evidence I consider intermediate filtration to be not only unnecessary but also harmful because (1)

it removes considerable quantities of virus giving a vaccine of inferior immunizing capacity and (2) it disturbs the regularity of the reaction making the course of the inactivation unpredictable

BIBLIOGRAPHY

Gard S Ciba Foundation Symposium on the Nature of Viruses p 123 London Churchill 1957

Haas R Korner L Dostal V and Schweinsberg H Ztschr Hyg Infkrankh 143 490 1957

Lycke E Arch Virusforsch 7 483 1957

Ross A F and Stanley W M J Gen Physiol 22 165 1938

Schwerdt C E and Schaffer F L Ann New York Acad Sc 61 740 1955

Taylor A R and McCormick M J Yale J Biol & Med 28 589 1955

Timm E A McLean I W Jr Kupsky C H and Hook A E J Immunol 77 444 1956

Wesslen T and Dinter Z Arch Virusforsch 7 394 1957

Wesslen T Lycke E Gard S and Olin G Arch Virusforsch 7 184 1957

Dr Smadel I believe that in the manufacturing of polio vaccine we have completed another era in the viral vaccines which in many respects is comparable to that of a decade or so ago when the yellow fever vaccines were first made What have we learned from polio vaccine that will be incorporated at least in the thinking in any viral vaccines made in the next decade or so? There are 5 points to consider

1 Mass tissue-culture methods can be used successfully for the large scale production of a vaccine for human beings We can confidently anticipate that many of our viral vaccines of the future will be produced in a similar manner

2 We are aware that commercial vaccines can be made which are relatively rich in viral antigens and poor in nonviral proteinaceous material Moreover the means are at hand for increasing the former and decreasing the latter

3 The transformation of safety testing of a killed vaccine from an art to a science has been hastened by measures incorporated in the polio vaccine production since the spring of 1955 All future tests on materials made from potentially hazardous agents will be based on more sophisticated statistical concepts than those generally applied earlier Moreover the quantity of materials employed in safety tests of new vaccines is unlikely to be much less than the equivalent of those now used in polio procedures The idea of obtaining and utilizing for safety tests carefully standardized cultures or animals having a high degree of susceptibility to minute amounts of live virus undoubtedly will be adopted in procedures for other viral vaccines

4 The idea is firmly established in the minds of many who have dealt with polio vaccine that greatest dependence can be placed on procedures in commercial production when each naked virus particle is exposed to the killing agent Aggregates of particles or protective coatings of any type on viral particles may impede inactivation Seitz filtration which is a required part of the inactivation procedure of the American polio vaccine presumably contributes to consistent inactivation by ensuring that all virus particles subjected to formalin are in an unprotected state Until an exact understanding is available of the means by which Seitz filtration accomplishes this it is unlikely that polio or other vaccines made in the United States from dangerous viruses of the small or medium size will fail to incorporate a filtration procedure

5 The large scale use of monkey tissue cultures has brought to light a new group of simian viruses some of which are related to previously known agents affecting man As pointed out by Dr Bodian the problems raised by these viruses have been solved by the manufacturers in the production of polio vaccine and these same solutions no doubt will apply to other killed vaccines made from such starting material However the simian agents will create additional problems for those who plan to make live attenuated viral vaccines from monkey kidney tissue cultures

Dr Bodian has also touched upon the matter of the Mahoney strain The idea is a sound one that an avirulent organism is better for making a killed vaccine in case one has an accident than is a virulent one But as he also pointed out it is as important to have an immunogenic material as it is to have one that is not hazardous

Dr Bazeley Wide scale immunization against polio was commenced in Australia at the beginning of June 1956 This program was undertaken without preliminary field trials The wide

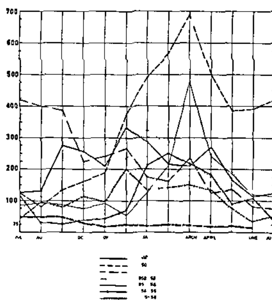


Fig 60 Incidence of poliomyelitis in Australia 1949 1957 (cases as reported by State Health Departments)

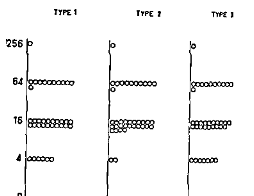


Fig 61 Serum antibody response in 38 school children previously negative to all 3 types (*Titers were obtained with vene* puncture blood the serum being expressed from the clot Roller culture technic essentially as described by Younger The dilution given is that of the serum itself This was in 0 25 ml of Hank s solution and mixed with the test virus dose also in 0 25 ml of fluid This 0 5 ml mixture was allowed to stand on the bench 30 minutes and added to the roller culture tubes in which the nutrient fluid (199) volume was 3 0 ml Trypsinized monkey kidney cells incubated 7 days were used)

experience with polio vaccine in the United States before that time was taken as a sound basis on which the program should be carried out in Australia As a result of this policy school children of all ages commenced to receive their first doses in the first days of June 1956 The rate of immunization was maximum from the outset

It was decided initially that the age group 0 14 years inclusive consisting of almost 2 500 000 children out of the total Australian population of 9 500 000 should be eligible for vaccination A 2 dose immunization for about 2 000 000 children within the first year of the program was the target

The program was in fact achieved Now at the end of the first year of immunization 2 012 000 children have received the first 2 doses of vaccine Booster doses for these children will be commenced in August or September (see Table 25) The latter phase is expected to be completed early in 1958

Under the circumstances in which the Australian program developed the surveillance system as a device tended more toward securing information about the vaccine s safety than providing a test of its efficiency as an antigen Particularly was this important in the first year of use of the vaccine The safety factor was a matter much in the thoughts of the adult population at that time Consequently the surveillance figures obtained must be evaluated largely from this point of view It was not intended at the outset that the program would in effect be a trial of the immunizing efficiency of the vaccine—this point really not being in any doubt

Table 25 Polio Immunization in Australia 1956 1957

Estimated population	9 300 000
Estimated number of children 0–14 years inclusive	2 470 000
Number of children given first 2 vaccine doses (June 1 1956—May 31 1957)	2 012 000

Notwithstanding this limitation certain figures have emerged from the first year s experience with immunization and these are shown in Table 26 Taken in relation to Figure 60 which shows in graph the epidemic history of poliomyelitis in Australia during the last 7 years it

Table 26 Polio Relative to Commencement of Vaccination in Australia June 1 1956 to May 31 1957

Among 2 012 000 Vaccinated Children	Expected	Actual
Polio cases less than 7 days after first dose	8	3
Polio cases between 7 and 28 days after first dose	25	4
Polio cases after second dose at any time during period	400	)
Polio cases for Australia (whole population) 12 month period (July 1 1956 to June 30 195)	2 253	224 (335)†
Polio cases for Australia (whole population) 5 month (January 1 1957 to May 31 1957)	1 2 4	71 (105)†

E pc te l figur ba d n n id n f pol d ng the p u year

† F t figur — ses pt d by Su lla Ce n t te Seco d fi u — tual n t h at n f m State a th c

seems clear that the vaccine has markedly lowered the incidence of poliomyelitis during the first 12 months of its use At no time in the past 12 years has the incidence fallen to such dramatically low levels as since the beginning of vaccination

The vaccine used in Australia was designed on the basis of experience acquired with Dr Jonas E Salk at the Virus Research Laboratory Pittsburgh U S A throughout its period of development The preparation and the performance of Reference Vaccine A which originated from his laboratory was the basis for planning the vaccine to be used in Australia Early work with Vaccine A had showed that the Type 1 component was not equally antigenic with the Type 2 or the Type 3 components in terms of uniform antibody response levels in the human subject and in monkeys Vaccine A was prepared by mixing equal parts of inactivated single virus type pools similarly prepared otherwise An adjustment was made in our case whereby a twofold concentration of the normal Type 1 component relative to Types 2 and 3 was achieved The vaccine used in Australia thus bears an original tissue-culture liquid volume relationship of 2 1 1 for each of the three types respectively all other factors being the same In this ratio the Type 2 and 3 components are considered to be at least equivalent in strength to the Type 1

In view of the over all powerful immunizing value of Reference Vaccine A and also the need to produce a vaccine of reasonably small volume for injection purposes a dose of 0 5 ml was considered likely to be satisfactory in producing reasonably good immunity Two intramuscular doses were given in the primary phase 1 month

Table 27 Comparison of Vaccine Potencies Virus Assay Relative to that of Vaccine A * Monkey Antibody Production Relative to that of Vaccine A†

	Potency Based on Virus Assay			Potency Based on Serology		
Vaccine Batch	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
6	1 2	0 8	0 6	1 2	0 6	0 8
7	1 6	0 8	0 8	2 0	0 8	0 8
8	1 ?	0 5	0 6	1 5	0 7	1 6
9	?0	0 6	0 8	1 4	0 8	0 5
10	?0	0 6	0 8	1 3	0 7	0 7
11	2 5	1 2	0 6	5	0 8	1 1
12	2 0	1 2	0 6	2 2	1 2	1 2
13	1 3	1 0	0 6	1 6	1 3	1 5
14	1 6	1 0	0 6	2 3	1 4	1 3
15	1 6	0 8	0 6	2 5	1 5	1 5
16	1 6	0 6	0 6	2 0	0 9	1 4

* Vi u a ay t expre sed a poter cy by t king the r thm t c at betw en the ru or ntr t n of o e flu d a d an the tand d o In th s ca e Va c A o g n l ru n nt t ha e be n used tand d te t l n lle t be These figu e we obt ed u g 20 t b at h lf log dil t t the s flu l t vec e 50 pe ent nd p t

Exampl	Reference Vaccine A	Batch 6	Potency Batch 6/Vaccine A
Type 1	10^{8}	$10^{-8\ 7}$	1.2
Type	$10^{7\ 9}$	10	0 8
Type 3	$10^{\ \ 9}$	10^{7}	0 6

Vot All nt s l ted to pu al alu of N I H Sta d a d Vi T8 (1 5) (Typ 1)

† M nkey rum pot ncies re obtai l by mpa i g g ometr m an of a t body esponse titer to gr ded d es of a c ne g n u ng Refe n e V c n A a a ta d rd Th geometric means p o led sl pe figu es that co ld be mpared mathematic lly w th othe Th batch pot y g en i th rati f th new a cine wh n mp d i th Ref n e Vac ne A by th se mean

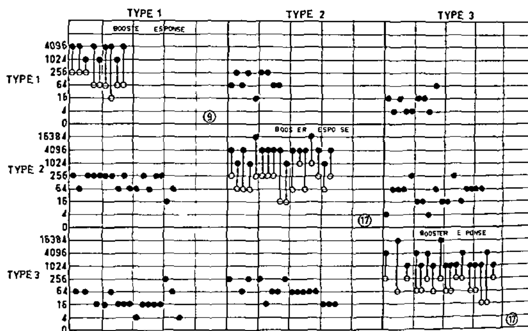


FIG 62 Antibody response in 43 school children with previous antibody to 1 type only (initial titers determined as in legend for Fig 61) Two doses 4 weeks apart vaccine batches 1 and 2

apart with the booster dose to follow approximately 12 months later The observations with children (recorded in Fig 61) showed that the composition of the Australian vaccine was capable of stimulating an equal response to all 3 virus types in triple negative antibody children This dose size also produced a generally high antibody level in a high percentage of subjects

When the potency of a number of batches of vaccine used in the campaign was examined by means of the monkey antiserum tissue-culture test system it was found that in general the levels established with Reference A had been equaled or surpassed with many consecutive batches of the vaccine In addition however these potencies appeared to be well related to the original virus concentrations of the vaccine before inactivation (Table 27) It seems reasonable to conclude from this work that a comparative relationship exists between the virus concentration a fluid has before inactivation and its final immunizing potency

The assay of virus fluids may well prove to be equally reliable or superior to the serologic test for immunizing potency It will always be difficult to employ and test a sufficient number of animals to produce serologic values of equal statistical significance to those readily obtained with a sound virus assay Reliance on virus assay of course is dependent on a constant inactivation procedure which we have always been able to follow i e 10 days of inactivation with 1/4 000 formalin added the fluids being held at 36 C

Field response of the vaccine with groups of children has been studied Using a roller-culture technic for serologic determinations and screening the groups very thoroughly it was found that only 10 12 per cent of some 681 children of all school ages examined could be regarded as completely free from demonstrable antibody to each of the 3 types of polio virus before vaccine doses were given This low percentage of triple zero antibody children was a surprise

The bulk of the children thus already possessed 1 or more antibodies Studying the response in those with a single prior antibody the vaccine dose produced levels that were high or low according to the level of the pre-existing antibody (Fig 62)

Some indications of the degree of boost provided by a pre-existing heterologous antibody with a subsequent single vaccine dose arose out

TABLE 28 SERUM ANTIBODY RESPONSE IN SCHOOL CHILDREN INTRAMUSCULAR INJECTION OF 0 5 ML OF VACCINES BATCHES 1 2 OR 11 4 WEEKS APART

Geometric Mean Titers In			
(a) Triple Negatives	Type 1	Type 2	Type 3
	20 65	23 73	19 92
(b) Double Negatives†			
1 Type 1 present		101 6	10 1
2 Type 2 present	133 6		33 4
3 Type 3 present	2 8	64 0	
Booster Response to Type Already Present			
4 Final Response	2 580 0	3 384 0	2 136 0
Pre-existing Titer	87 1	133 6	96 4

Sam   F g c f2
†M f m h wn n F gu 6

of the above work (Table 28) The mean response achieved was perceptible but not to be compared with the amount of homologous booster response noted It was particularly pleasing to note the uniformity of the booster response to pre-existing natural homologous antibody together with the high antibody levels achieved At a later date these will be compared with the nature of the response when vaccine stimulated antibody alone is present

Whether the degree of immunization reached in Australia by the above procedures is needed to control poliomyelitis is of course not clear from the above results However the program has proceeded in a very satisfactory manner

We have developed a feeling of some security in this matter by the use at the outset of a moderately concentrated polio virus antigen of fairly uniform composition prepared with constant procedures

BIBLIOGRAPHY

Salk J F Vaccinat on against paralytic poliomyelitis performance and prospects Am J Pub Health 45 575 1955

——— In press

Salk J F *et al* Antigenic activity of poliomyelitis vaccine undergoing field test Am J Pub Health 45 151 1955

Younger J S Monolayer tissue cultures II Poliomyelitis virus assay in roller tube cultures of trypsin-dispersed monkey kidney Proc Soc Exper Biol & Med 85 527 1954

DR GEAR After preliminary studies initiated in 1952 in collaboration with Dr Thomas Weller who it may be remembered with Professor Enders and Dr Robbins was responsible for the fundamental discovery that polio virus would grow in tissue cultures of non nervous human tissue production was begun in 1953 The method of production with minor modifications was that prescribed by Salk We have preferred the Brunenders strain to the Mahoney strain as a representative of Type 1 The formaldehyde inactivation has been supplemented by treatment with ultraviolet light

As with most others engaged on a similar task we have had our difficulties And in solving them we would like to acknowledge the generous help that we have received from our friends in the United States Great Britain Denmark Sweden and more recently Australia

We have encountered all the difficulties in production especially in filtration described by Dr Bodian In safety testing too we have met with them Dr Malherbe and his team have been particularly worried by the occurrence of simian agents in the tissue cultures This difficulty has been solved by preparing trypsinized cells from single kidneys In the potency tests after trying baboons and monkeys we have preferred the guinea pig test as described by Sven Gard We learned the hard way and it is gratifying now to report that production is going more smoothly

So far about 500 000 children have been vaccinated in South Africa Reactions to the vaccine have been few and none were very serious The campaign was carried out in the face of the worst epidemic that South Africa has exper

enced The occurrence of coincidental cases and they are bound to occur has given rise to much anxiety on occasion The protection conferred by the vaccine seems to be similar to that achieved in the United States where as we have heard from Dr Langmuir about 75 per cent of those children who otherwise would have contracted paralytic poliomyelitis do not This is a notable achievement But what of the remaining 25 per cent who are not protected? Can we do anything to protect them? That is an important outstanding question

Then how should we deal with an impending epidemic? From Dr Langmuir s paper we learn that vaccination with the killed vaccine in the face of such an epidemic did not bring it to an end That was our experience also Could it be brought to an end in any other way? This is another important question to which an answer is urgently needed because in the immediate future many countries will be faced with impending epidemics

And finally it is clear from our findings and also from the more extensive studies in the United States that vaccination with inactivated vaccine does not prevent infection This may be a happy circumstance because it does not deny the children who are vaccinated the protection which results from natural infection

PROF DR HAAS Dr Bodian has stressed the importance of filtration for the production of vaccines particularly those which are free of viruses which are likely to multiply So far in Germany we have used poliomyelitis virus grown in trypsinized cultures which had been obtained from rhesus monkeys As far as the filtration is concerned from the beginning we used a certain number of Seitz filters or a certain number of combinations of Seitz filters and membrane filters Quite apart from the asbestos contents of the levels there is nevertheless a certain amount of loss of the virus These however may be at least reduced as far as that loss is concerned by using a proper gradation of filters so that this does not really play an important part and we were able to use a certain combination of filters so that we really did not have any great losses

According to our experience we feel that you can also obtain better results by centrifugation and this may bring about in certain cases an interruption of filter losses During the inactivation larger losses may occur, in fact larger than before or after activation Dr Gard in his last figure has already referred to our experience and has shown that our values are quite close to his own findings Nevertheless I should like to point out that the main difficulty resides in the fact that in connection with the safety of vaccines we have to say something about concentration whereas we do not really measure the concentration exactly and accurately Consequently the important point of view is probably an approximation rather than an accurate figure Dr Gard referred to the immune biologic processes and he spoke about other effects that may take place The aluminum hydroxides have been used by us or at least we have examined this technic and found that by adding aluminum hydroxide in small quantities we were able to obtain effects which increase immunity at least in tests with mice

Vaccines with aluminum hydroxide added enabled mice to resist intracerebral Type 2 infection better than mice immunized with vaccine prepared without aluminum hydroxide We were able to demonstrate in the rabbit that the addition of aluminum hydroxide may bring about a slowing down of the neutralizing effect However the concentration came to higher titers and after several months of observation we found that it was always higher than the concentration of antibodies which had been obtained from animals and had been immunized without the addition of aluminum hydroxide

PROF DR HALLAUER The prevention of paralytic poliomyelitis through uncontrolled mass vaccination is a problem which in the opinion of many public health authorities has been solved for all practical purposes excepting possible technical improvement Therefore discussion concerning the mode of action and critique of the efficacy of the vaccine is considered in certain quarters as obsolete However since Salk himself in earlier publications and again in his paper today has examined the theoretical basis of polio vaccination and as a result has come to the optimistic conclusion that his procedure not only duplicates but surpasses the protection effects of naturally acquired immunity it seems permissible for a neutral observer to comment on this question

1 It is obvious that no fundamental differences exist between Salk vaccination and other vaccination procedures which employ nonliving antigenic material with respect to preparation of vaccine method of immunization and development of antibodies As a matter of fact no radically new observations were discovered in the perfection of Salk s vaccin and its mode of action follows without mystification well-established principles The only surprise perhaps was the discovery that inactivation of polio virus through formalin apparently does not take place as a first order chemical reaction which in turn makes it impossible to eliminate the very last active virus particles from the preparation

2 Because of these facts there is no reason to assume that the Salk vaccination should not be subject to the same inherent limitations of efficacy which characterize all similar vaccination procedures with the added importance that failures in this field are even less acceptable because of the slight risk of exposure As is well known the protective effect stands in some correlation with the antibody level in the blood stream the persistence of which can be guaranteed only by periodic revaccination or subsequent subclinical infection

3 To what extent such humoral immunity is actually protective does not depend on the immunization procedure but is governed by the particular properties of the infectious agent its tissue tropism toxin formation etc The assumption has been made that the antibody barrier in the circulation is sufficient to intercept polio virus on its way to the CNS because of the further assumption that most strains utilize the hematogenic pathway of infection exclusively Whether this apodictic pronouncement is correct must appear doubtful if for no other reason than because of the fact that Bodian himself has documented with equal persuasiveness both neurogenic and viremic channels of virus transfer in his publications of 1940 1941 and 1952 1955 It may be added that maximal circulating antibody levels do not have an immediate sterilizing effect but may permit the persistence of active virus in the blood and the internal organs for at least 8 to 10 days a fact well established for fowl plague yellow fever foot and mouth disease virus and others

4 A comparison of naturally acquired immunity with the Salk vaccination shows fundamental differences with respect to the mechanism of immunity and the degree of protection The superiority of living over nonliving antigens depends less on possible advantages of quantity or quality than on the pattern of distribution in accordance with specific organ or tissue affinities In the process of infection it is those tissues that function as portal of entry avenues of dissemination and finally as a target of the lesion which are actively immunized with the production of histogenic immunity Under these conditions the liberation of humoral antibodies appears as a secondary diagnostic reaction and the immunologic barrier lies in these same tissues which are directly involved by the infectious process and not in the blood Natural immunization against poliomyelitis probably is protective as a rule because of the development of a tissue immunity of the lymphatic apparatus of the gastro-intestinal portal of entry The possibilities of effective local immunization with killed antigen against enteric organisms were demonstrated successfully some 50 years ago by Metschnikoff and Besredka In view of these facts it seems not unlikely that enteric application of formalinized polio vaccine may succeed better than intramuscular injection of the antigen Such procedure might give a more durable local immunity and the problem of the mode of transfer of the virus to the CNS would become essentially irrelevant with this form of vaccination

Dr Murray The data presented by the first three papers here today have shown that the field experience with poliomyelitis vaccine indicates that poliomyelitis vaccine as now used continues to be a highly effective agent in protecting against paralytic disease

However scattered fragmentary observations have suggested that commercial vaccines have not been eliciting antibodies in an appreciable proportion of triple negative children who have received 2 injections

With such observations in mind a controlled study of a number of commercially produced vaccines was undertaken in order to determine the antibody response in triple negative children The experimental design called for the inoculation of infants—these were 6 months to 3 years of age—in 4 widely separated cities Triple negative subjects of older age groups were

not available Two injections were given 1 month apart and a bleeding was taken 1 month after the second injection It is planned to take a further bleeding after a third injection Assays were done in the laboratory animals using both monkeys and chicks which we are using now for comparison

So far complete results are available only through the second injection in the case of only 4 vaccines of the 9 under study The scatter of antibody responses is shown in Figure 63

An attempt was made to compare these responses with those which were obtained in the 1954 field trial where of course 3 injections were used It will be remembered that the correlations obtained in the field trial between protection against disease and antibody responses enabled a classification of the vaccines used at that time into several categories from good to poor The results in the present study were compared with those in the published reports of the field trial in terms of percentage conversions at 1/16 or above The figure 1/16 was chosen because the groupings were different in the two studies These results are shown in Figure 64 Two additional vaccines E and F on which partial results are available are included as a matter of interest These are the shaded bars Vaccine A and A′ were identical except that Vaccine A was used in one area and Vaccine A in four It will be seen that in the case of the vaccines for which we have complete information that responses to Type 2 were in general equal to or better than those encountered in the field trials The Type 1 responses were generally equivalent to those classified as fair to moderate in the field trials while the Type 3 antibody responses of these vaccines compared rather unfavorably with the vaccines used in the field trial falling into the poor to fair categories

In general the animal tests correspond to the results shown in children and the figures obtained in monkeys correlate very well with those obtained in chickens

The present data if taken out of context with the other information reported today might be considered discouraging However all things considered the situation appears to be the following

Two injections of ordinary commercial vaccine leave a high proportion of triple negative children without detectable Type 1 and Type 3 antibodies Nevertheless protection against paralytic disease is induced by this procedure And it is worth noting that preliminary observations of others indicate that completion of the course of immunization by a third injection of commercial vaccine induces antibodies to all 3 types in the majority of children However the present results do re emphasize the desirability for increasing the immunizing capacity of the Type 1 and the Type 3 components of the vaccine

I would remark about Dr Bradford Hill's

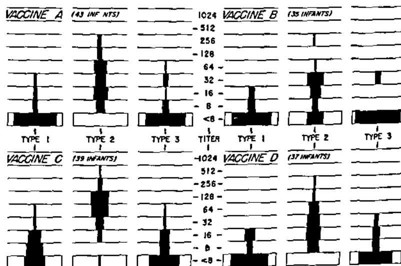


FIG 63 Polio vaccine lot potency investigation

comments relative to 2 versus 3 injections—we would all feel happier if we could show that they actually had antibodies after 3

Dr Penttinen I will show one chart (Table 29) showing the potency of 2 lots of polio vaccine used in Finland as measured by antibody production in vaccinated children As you will see from the table the 1954 experimental vaccine used in Finland had a poor antigenicity As regards Type 1 you see that only 12 per cent developed antibodies They were children who did not have any antibodies before the vaccination and also the control group indicated that the increase was not due to vaccination but to the polio epidemic It was completely ineffective against Type 1 Type 2 results and Type 3 results are better

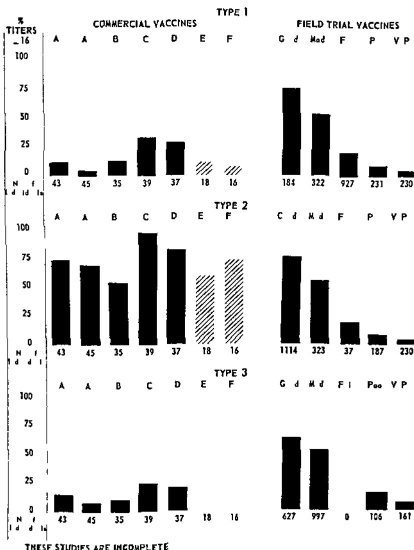


Fig 64 Comparison of human antibody responses for several current commercial poliomyelitis vaccines and field trial vaccines of varying effectiveness

TABLE 29 RESULTS WITH 1954 AND 1957 VACCINE SECOND BLOOD SPECIMENS COLLECTED 2 WEEKS AFTER LAST INJECTION ONLY NEGATIVES IN THE FIRST TEST PRESENTED

VACCINE	TYPE 1			TYPE 2			TYPE 3		
	NUMBER TESTED	NUMBER +	PER CENT	NUMBER TESTED	NUMBER +	PER CENT	NUMBER TESTED	NUMBER +	PER CENT
1954	134	16	12	92	31	34	63	16	25
1957	46	25	54	48	37	77	48	25	52

Control group indicates that the increase was due to polio epidemic

In 1957 the vaccine is much better The antibody response against Type 1 is 54 per cent Type 2 77 per cent and Type 3 52 per cent They all are children who did not have any antibodies before the vaccination

It is possible that the 1954 experimental vaccine was damaged by merthiolate or due to shipment In 1957 commercial vaccine as you see was much better although as regards measurable antibody formation not complete when children with no antibodies before vaccination were in question

PROF DR PETTE On the basis of experimental studies conducted at the Institute of the Foundation for Research in Spinal Paralytic Poliomyelitis and Multiple Sclerosis I would like to make the following comments

1 In his paper Dr Salk concerned himself with the question of Type 1 hyperreactivity in Type 2 immunity Figure 65 shows the results of personal investigations Of 150 patients who had developed paralytic or aparalytic meningitic poliomyelitis following an infection with Type 1

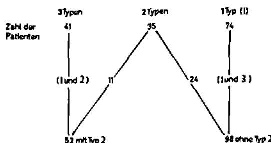


FIG 65 Antibodies against poliomyelitis after illness with Type 1 (150 patients—paralytic aparalytic meningitis)

polio virus 41 exhibited antibodies against all 3 types 35 exhibited antibodies against 2 types and 74 exhibited antibodies against 1 type Type 1 Of the 35 patients with antibodies against 2 types 11 had antibodies against Types 1 and 2 and 24 had antibodies against Types 1 and 3 Altogether therefore 52 of 150 patients had before infection with Type 1 antibodies against Type 2 98 not exhibiting such Type 2 antibodies If the paralytic and nonparalytic cases are considered separately we find that this ratio remains the same From this we conclude that the immunobiologic hyperreactivity to Type 1 that Dr Salk assumes to be present in Type 2 immunity and the protection resulting therefrom should not be rated too highly

2 Immunization experiments with viruses of the EMC group in rabbits and guinea pigs have shown that here too the antibody content of the serum is limited in its dependence on the introduction of active antigen No specific antibody maximum can be increased by further administration of antigens

3 We have investigated experimentally the question of whether the increase in antibody titer after polio vaccination could be a nonspecific anamnestic reaction Following encephalography with air smallpox vaccination diphtheria toxin and tetanus toxin inoculation in 30 subjects no increase in polio antibodies was noted

4 We agree with Dr Bodian that tissue cultures to determine the presence of any virus in the vaccine can be more useful than the monkey test but share his view that we cannot dispense with the latter particularly in view of the desirable maintenance of continuous safety controls Evaluation of neurohistologic changes requires however especially extensive experience in neuropathology and the most critical judgment



Fig 66 Encephalomyelitis in a monkey



Fig 67 Encephalomyelitis in a monkey

5 With regard to Dr Langmuir s paper even if it is true that neuro allergic reactions are relatively rare following polio vaccination since severe forms of postvaccination encephalomyelitis seem to have been observed in only isolated cases more attention than heretofore should be given to the *formes frustes* of allergic encephalomyelitis i e those cases in which collapse occurs immediately after the second or third injection It should not be forgotten that in cases of collapse lasting for several hours cerebral circulatory disturbances in the gray substance of the central nervous system particularly in the cerebral cortex can develop From experience with other types of disturbances that occur following infection and vaccination anaphylactically or with disturbances of the delayed type we know that su h children can later undergo psychic changes or also react with epileptic attacks It is the clinician s duty to draw attention to this possibility Finally it is generally ac epted biologi knowledge that repeated injections of foreign protein into an organism can lead to allergic reactions not only in the central nervous system but in other organs as well

Figures 66 and 67 will serve to demonstrate an instructive experimental finding yet one which of course should not be generalized Of 6 monkeys inoculated with Salk vaccine 1 of the animals became sick a few days after the third injection exhibiting myelitis Histologic investigation revealed the classical picture of a perivenous encephalomyelitis in addition to a nodular pulmonary tuberculosis which pathogenetically speaking must have been a decisive factor in the development of the encephalomyelitis An interesting fact is that the antibody titer of the sick monkey was considerably higher than that of the 5 other animals vaccinated This basically important experimental finding in the animal indicates that one should proceed with reserve in considering polio vaccination for children suffering f om a chronic infectious disease especially tuberculosis The same is true of children who have a marked tendency to severe allergi reactions In this onnection the latest finding reported by Uehlinger of Zurich (*Schwei med Wchnschr* p 813 1957)—that of a case of anaphylactic reaction following poliomyelitis vaccination in a 4½ year-old child who developed a condition resembling Landry s paralysis 3 days after the second injection and died—certainly merits particular attention

In closing I mention the results of antibody determinations in a number of children who had not shown any antibodies against one of the 3 polio virus typ s (so-called nuis) prior to inoculation Two weeks after the second administration of the accine the antibody titer was found to be very good against Type ? good against Type 1 and fair against Type 3

Prof D Stuart Harris My chief comment as one who has been concerned in the question of field trials relates to the great debt which we all owe to tho e who planned and executed the Francis field trial in the United States We acknowledge this because the scientific impeccability of this trial enabled others of us faced by the problem of the introduction of the use of the

Salk vaccine to concentrate on certain questions which appeared to require additional answers I hope that our experience in Great Britain in relation to the protection in the under school age child and also with the Brunenders virus as an alternative to the Mahoney strain will be useful

To turn to the problems of the future pride of place in my view should undoubtedly go to the question of how does the vaccine work? The remarkably small amount of virus content in the vaccine contrasts with its efficiency as a method of inducing antibodies Also as already mentioned this afternoon after use of the vaccine one can still obtain infection of the alimentary tract even though paralysis does not occur And this fact contrasts with the effect of natural infection It is remarkable perhaps that nonparalytic disease is not apparently affected by the vaccine though the ECHO and the Coxsackie viruses undoubtedly interfere with the assessment of the vaccine against this type of disease In the British trial there was some reduction of *nonparalytic disease in cases from which actual* poliomyelitis viruses were obtained The evidence was just suggested One can only speculate concerning this question of whether the vaccine induced antibody prevents blood stream invasion or that it operates at a neuronal level either at the periphery or within the areas of the blood brain barrier These are questions which the immunologist must solve no doubt in the future and also additional questions concerning the duration of immunity and the hyperreactive state

Unquestionably one of our future problems is going to be How can a population be induced to accept vaccine to the required level for the complete control of the disease? Nowadays one third of all of our cases of poliomyelitis in Britain occur in those over 15 years of age How can we persuade adults to be immunized to the fullest possible extent during the early phase of a mass campaign? This is a matter which undoubtedly will be solved in different countries by different ways but in a country such as Great Britain where mass anything is questioned it will require a considerable effort to persuade adults to be immunized Perhaps it may yet be found that the disease incidence will wane in other groups once the most susceptible infants *and children* have been immunized

The third question for the future seems to be What should be the earliest age at which vaccine should be administered? In Britain the methods and the timing of inoculations with antigens such as diphtheria and whooping cough are being revised in order to aim at the prevention of provocation poliomyelitis It is clearly advantageous to give the vaccine at the earliest possible age compatible with an adequate *antibody response Is 6 months too early because* maternal antibodies may still be present or would it be safer to give the vaccine later? In view of Dr Langmuir s figures of the continued high attack rate of poliomyelitis in infants under 1 year it seems essential to give the vaccine at as early an age as possible This question probably requires laboratory study

Finally several people have mentioned the problem of the relative power of protection of 2 doses as compared with 3 doses of vaccine Whatever may be said about the antibody response this is really a matter which requires further field study in order that complete verification can be obtained

Dr Tobin Dr Bradford Hill has said that the Mahoney strain has been replaced by the Brunenders attenuated strain in the production of British vaccine The Type 2 and Type 3 strains have remained the same as those used by the Americans He has also said that the British vaccine has given good protection It is not surprising then that it is also antigenic

Figure 68 shows the geometric mean titers *obtained with the 3 batches of* British vaccine produced by Dr Wood and his colleagues at Glaxo and which were used in the British field trial of last year They are compared with a good American commercial vaccine kindly provided by Eli Lilly and Company In the top line of pillars is the Type 1 response The antigenic response produced by the Brunenders component compares well with that produced by the *Mahoney one contained* in the original vaccine The Type 2 and 3 components being produced by the same strains as the American vaccine have also given comparable results These are the results in monkeys

We have also obtained results with the British vaccine in triple negative children These children I think are definitely triple negative because the methods used for estimating the antibodies is very sensitive Although we have been using roller tube technic we have kept the

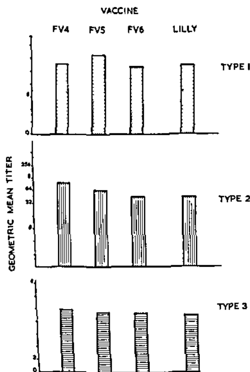


Fig 68 Geometric mean titers obtained with 3 batches of British vaccine and 1 American vaccine

virus and the serum dilutions in contact for 3½ hours at 30 C as this seems to increase the sensitivity of the test

The children who were triple negative were between the ages of 1 and 9 and we have divided them into those over 5 and those under 5 as the antigenicity in these 2 groups was of interest (Fig 69) The Type 1 2 and 3 components have all given a good response in both age groups *In spite of the championship of* the Mahoney strain by Dr Bodian and Dr Smadel the Brunenders strain is not so bad for producing killed polio vaccine However the introduction of an attenuated strain has made us alter our safety testing procedures which are almost the same as the American procedures in one respect This strain if inoculated into cynomolgus monkeys in doses of 100 000 to 1 000 000 tissue-culture doses will produce viremia in nearly all monkeys but no invasion of the central nervous system occurs As you can see it is attenuated

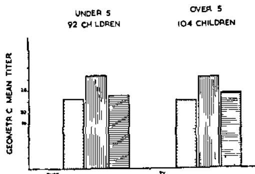


Fig 69 Geometric mean titers obtained in triple negative children

However with the simultaneous injection of cortisone 10 to 20 tissue-culture doses will regularly produce infection in cynomolgus monkeys with degree of involvement of the central nervous system depending on the dose of cortisone For this reason we are retaining the monkey test in the safety test of our vaccine also keeping in mind of course that we have to test for Types 2 and 3 But the sensitivity of these monkeys under the treatment of cortisone in direct titrations is much of the same order as one gets with Mahoney strains in normal monkeys

However with this Brunenders strain there is some inhibition when a vaccine is inoculated *simultaneously and we are not quite yet certain* of the dose to be used in order to get the required sensitivity as compared with the amount of vaccine tested If any reversion to more virulence does occur and live virus may have been missed in a monkey the monkey test itself will automatically become more sensitive and will act as a second safeguard in case for some unknown reason the tissue-culture tests do break down

BIBLIOGRAPHY

Bodian D Am J Hyg 64 97 1956
Eklund C M Bell E J and Hadlow W J Am J Hyg 64 85 1956
Enders J F Weller T H and Robbins F C Fed Proc 11 467 1952
Gear J South African M J 30 587 1956
Report Brit M J 1 366 1957
Sabin A B Ann New York Acad Sc 61 1050 1955
Syverton J T Brunner K T Tobin J O H, and Cohen M M Am J Hyg 64 74 1956

Vaccination Against Poliomyelitis

TUESDAY MORNING, JULY 9, 1957

Chairman

DR JAMES H S GEAR
South African Institute
for Medical Research
Johannesburg

Speakers

DR HILARY KOPROWSKI
The Wistar Institute
Philadelphia

DR ALBERT B SABIN
Children's Hospital Research
Foundation
Cincinnati

DR JOHN P FOX
Tulane University
School of Medicine
New Orleans

DR A M M PAYNE
Section of Endemo Epidemic Diseases
World Health Organization
Geneva

Discussants

DR GORDON C BROWN
University of Michigan
School of Public Health
Ann Arbor

DR M P CHUMAKOV
Poliomyelitis Research Institute
Academy of Medical Sciences of
the U S S R
Moscow

PROF DR G W A DICK
Queen's University
Department of Microbiology
Belfast

DR P V GHARPURE
Polio Research Unit
Indian Council of Medical Research
Grant Medical College
Bombay

PROF A GIOVANARDI
Institute of Hygiene
Milan

DR NATAN GOLDBLUM
Ministry of Health
Virus Laboratory
Jaffa

DR GEORG HENNEBERG
Robert Koch Institute
Berlin

DR DOROTHY HORSTMANN
Yale University School of Medicine
Section of Preventive Medicine
New Haven

PROF PIERRE LEPINE
Institut Pasteur
Paris

DR VITTORIO MONACI
Institute of Hygiene
Milan

PROF NILS OKER BLOM
Municipal Bacteriological Laboratory
Helsinki

DR JOHN R PAUL
Yale University School of Medicine
Section of Preventive Medicine
New Haven

DR JONAS E SALK
Virus Research Laboratory
University of Pittsburgh
Pittsburgh

Vaccination with Modified Active Viruses

DR HILARY KOPROWSKI

The careful and patient evaluation of immunization with attenuated living polio viruses during the past 7 years now has reached a point where large mass clinical trials have to be taken into serious consideration If this method of immunization which may lead to the eradication of poliomyelitis virus as a constant menace to the world community is effective then there is all the more reason to be fully cognizant of all the factors involved in studying the problem

The purpose of this presentation is to examine critically the work done with the attenuated polio viruses now available which judging from all *experimental data are probably as good as* they ever will be and hence should be given a fair trial

Type 1 SM strain was originally attenuated by intraspinal passages in mice Only 3 human subjects received the material representing the 28th mouse brain passage of the virus (Table 30) *This strain has been passaged through 14* serial chick embryo tissue-culture transfers but because the 14th passage failed to show cytopathogenic properties for any of the tissue culture systems it was assayed on 5 alternate passages between monkey kidney tissue cultures and chick embryo tissue cultures were made in *order to obtain and maintain a demonstrable* concentration of the virus This 5th alternate passage was made into a large chick embryo pool called SM N 90 strain Its pathogenic properties for either the rhesus or the cynomolgus monkeys injected intracerebrally were found to be of very low order although occasionally *lesions were found in the central nervous system* of monkeys who apparently remained symptom free after the inoculations Thus we may assume that by passage through several generations of nonprimate hosts a population has been obtained still probably nonhomogenous which was predominantly characterized by decreased neurotropism for monkeys

At this point it was decided to apply the technic of purification of the strain introduced by Dulbecco *et al* by studying single plaque progenies The first plaque of the material was originally isolated by Dr Dulbecco and in my laboratory this material was further passaged through 4 single plaque passages before it was made again into a pool for study This subline *named SM 45 strain was submitted* to tests for neurotropism by intracerebral and intraspinal inoculation of cynomolgus monkeys and was found to be attenuated to a high degree as evidenced by very low intraspinal pathogenicity for monkeys

The history of the CHAT strain which is *summarized on the right of Table 30* is also of interest This line originated from the 4th serial human passage of the N 90 pool Fecal virus was plated out in monkey kidney monolayer and single plaque progenies were transferred through 3 additional passages We have obtained an indication of a remarkable degree of *attenuation In Table 31 you will* see the last 3 plaques examined and analyzed and you will notice the very low intracerebral/intraspinal pathogenicity of the plaques which are numbered 39 41 and 42 This was substantiated further by the absence of clinical signs of illness in 5 chimpanzees injected intraspinally with *this substrain and by the* lack or minimal presence of histopathologic lesions in the anterior cords of monkeys injected with this strain

Results of titration of the CHAT strain in monkeys inoculated with serial dilutions are shown in Table 32 The lack of pathogenicity after intracerebral inoculation is confirmed by *the absence* of histologic lesions in the central nervous system The histologic examination of spinal cords of monkeys injected with the large $10^{6\ 5}$ tissue-culture doses revealed an interesting phenomenon namely that the *paralysis* observed in 1 of the monkeys was caused probably by trauma since no neuronal lesions characteristic for poliomyelitis were observed In 2 other monkeys of which only 1 showed signs of paralysis after injection with $10^{\ 5}$ tissue-culture

TABLE 30 HISTORY OF THE ATTENUATION OF SM VIRUS (TYPE 1)

Adaptation to mice from monkey kidney (MK)
↓
1 Swiss plus 27 PRI m ce —————— 3 ch ldren*
↓
14 uccess e chick embryo (CE) tissue culture MK N 90 strain
↓
5 alternate MK CE tissue culture—CE seed pool —————— 53 adults
32 babies†
142 children and adolescents —————— → 4 serial human^
36 reported‡
20 reported§
Total 227 (including 14 fed by Dick *et al*)

Left branch (from CE seed pool):
↓
MK *plaque (by Dulbecco—August, 1955)*
↓
MK plaque
↓
MK plaque
↓
MK plaque
↓
Pool 42
↓
1st CE tissue culture Pool 45 SM 45 strain——babies and adults

Right branch (from 142 children and adolescents):
4 serial human^
↓
MK plaque
↓
MK plaque
↓
MK plaque
↓
MK plaque
↓
MK—CHAT Strain—babies and adults fed

MK = M k y k lney
CE = Cl k mbryo
* K p k *et l* P oc Soc Exper B l & Med 86 244 1954
† Kop w k *e l* J A M A 162 1281 1956
‡ K p w k *e al* J A M A 160 954 1956
§ K p w k *e al* Am J M Sc 232 3/8 1956
^ Di k *e l* Br t h M J N 5010 p 65 195
N l n T L *et al* To be publ hed

TABLE 31 SM CHAT PLAQUE LINE

Final Stool Conc (5 7)*
↓

Plq 1 I C 1/4† [1 Min]‡ I S 1/4 [1]	Plq 8 I C 0/4 [0] I S 0/4 [1]	Plq 9 (5 2) I C 0/4 [0] I S 0/4 [0] ↓	Plq 10 I C 0/4 [0/3] I S 0/4 [0]	Plq 11 I C 0/4 [0] I S 0/4 [0/3]	
Plq 13 (7 2) I S 2/5 [1] ↓	Plq 14 I S 2/5 [0]	Plq 15 I S 0/5 [0] ↓	Plq 16 I S 0/5 [1]	Plq 17 I S 3/5 [0]	
Plq 18 (7 2) I S 1/4 [1]	Plq 19 (7 7) I S 0/4 [1]		Plq 20 (8 2) I S 0/4 [0] I S 0/5 (Chimps) ↓	Plq 21 (7 0) I S 1/4 [1 Min]	Plq 22 I S 2/4 [1/3]
	Plq 23 I S 2/4 [0]	Plq 24 I S 0/4 [1 Min]		Plq 25 I S 2/4 [0]	Plq 26 I S 2/4 [3]
					Plq 27 I S 1/5
Plq 34 (8 2) I S 1/4 [0]	Plq 35 (7 5) I S 1/4 [1]	Plq 36 (7 5) I S 1/4 [0] ↓	Plq 37 (7 0) I S 0/4 [1]	Plq 38 (6 7) I S 1/4 [1]	
	Plq 39 (7 2) I C 0/5 [0] I S 0/5 [0]	Plq 41 (6 5) I C 0/4 [0] I S 0/5 [0]	Plq 42 (6 7) I C 0/5 [0] I S 0/4 [0]		

Plq = Plaque

Fig r s in ( ) = tit of irus in monk y k dney tissue culture as logs to ba e 10

† R ti of monk ys how g cl n c l s g s after I C (ntrac eb l) o I S (ntra pinal) inj ct n

‡ Figur in [ ] d c te numb f monkeys n which hist path logic exam n tion r e l d CNS le ns g [0] d nt to 0/4 nd at th t all 4 m nk y f ee of l ns [0/3] adj cent to 0/4 ind cat s that only 3 out of the 4 w ex mined for h tolog c l n nd no w fou d

TABLE 32 PATHOGENICITY FOR MONKEYS OF THE CHAT STRAIN OF TYPE I VIRUS

ROUTE	LOG OF TCD 0 INOC	PARALYTIC RATIO	RATIO OF CNS LESIONS	CELLULAR LOSS PER CENT CERV	CELLULAR LOSS PER CENT LUMBAR
Intracerebral	7 2	0/4	0/4		
	6 2	0/4	0/4		
	5 2	0/4	0/4		
	4 2	0/4	0/4		
Intrasp nal	6 5	1/4	0/3*	12	20
	5 5	1/4	2/4†	10	16
	4 5	0/4	0/4		
	3 5	0/4	0/4		

H t l gic x m n t n f 1 symptomat m nkey was n t p tf m d

† T umatic les n bser ed n b th n t n

doses of virus histologic examinations showed presence in minor degree of destruction of anterior horn cells at lumbar and cervical enlargements of the spinal cord The remaining results were negative

Investigations of properties of an attenuated strain of poliomyelitis virus to be comprehensive obviously should include examination of the virus after passage through the human intestinal tract In the course of years of observations it became apparent that the virus population multiplying in the alimentary tract of an infant may undergo some increase in neurotrop c properties but that by passage through the intestinal tract of an adult it does not Consequently monkeys were injected with fecal extracts orig nally obtained from infants fed this CHAT virus The results shown in Table 33 indicate that in the first case a pool made of fecal virus obtained at different time periods after feeding failed to cause paralysis or spinal cord lesions in monkeys injected either intracerebrally or intraspinally Total fecal output of the second case marked TA obtained during the entire period of alimentary infection was processed and the concentrate titrated in monkeys No paralysis was observed in monkeys injected intracerebrally and only slight paralysis

TABLE 33 FATE OF MONKEYS INJECTED WITH CHAT STRAIN OF ATTENUATED TYPE 1 VIRUS

SUBJECT	MATERIAL	DAYS AFTER INGESTION OF VIRUS	INTRACEREBRAL TCD INOC	INTRACEREBRAL PARALYTIC RATIO	INTRACEREBRAL LESIONS	INTRASPINAL TCD INOC	INTRASPINAL PARALYTIC RATIO	INTRASPINAL LESIONS
BO	Feces	3–6 10–13 17 20 24 27	3 4	0/5	0/5	2 7	0/5	0/5
T A†	Feces	1 26‡	3 7	0/4	N T	3 2	2/4	N T
			2 7	0/4		2 2	1/4	
			1 7	0/4		1 2	0/4	
			0 7	0/4		0/2	0/4	
G A§	5th T C	1 36‡	7 4	0/5		6 7	0/5	

B by 87 da old t th t me of t u a lm n tr u n

† B by 28 d y ld at th t me of dm n t at n

‡ T tal fec l v us outp t sed seed f r t cultu

§ B by 80 d y old at the t m f v u dm t at n

in one of the lesions was seen in each of the 3 monkeys tested intraspinally. To counteract criticism that concentration of the fecal virus was too low to reveal changes in the character of the virus, total fecal virus obtained from a third infant, marked GA on the table, was passaged through 5 monkey kidney tissue-culture passages in order to increase the titer of the virus. The results indicate that even large doses of the CHAT strain, after passage through human intestinal tract, failed to cause paralysis in monkeys.

Study of characteristics of another progeny of the SM Type 1 virus, the 45 strain, which was derived also through single-plaque passages (Table 30), is now demonstrated in Table 34. *In addition to the inoculum* also the total fecal virus output obtained during the period of alimentary infection from a 39-day-old baby was tested in monkeys. In order to raise the concentration of the virus, 4 additional tissue-culture passages were made and then used as inoculum. The results failed to show any increase in pathogenicity for monkeys inoculated intracerebrally. Two monkeys showed paralysis after intraspinal inoculation which does not necessarily indicate increased neurotropism, since at present there is no strain of attenuated polio virus available which is completely devoid of paralytogenic properties after intraspinal inoculation of monkeys.

The attenuated Type 3 polio virus also has been subjected to rigid tests after passage through the alimentary tract of infants. Originally it was derived from a stool of an asymptomatic child and purified through 6 single-plaque passages, as shown in Table 35. The brackets in this table refer to the histologic examination and the lesions found in the monkey's central nervous system. Fate of the virus following its administration to a 79-day-old infant is shown in Table 36. The original feces contained too little virus for the negative results to be of significance. However the 2 tissue-culture passages of fecal virus failed to show any increase in the paralytogenic properties of the virus even though large concentrations of infectious material were injected into monkeys either by the intracerebral or intraspinal routes.

The currently employed Type 2 polio virus has been derived from an infant who ingested one of the MEF1 substrain viruses. Because of lack of cytopathogenic properties of the original egg passaged strain, it was impossible to subject it to purification through isolation of progenies of single plaques. However, a passage through the intestinal tract of an infant yielded a virus which was "weakly" cytopathogenic but became more so in the course of repeated passages through tissue-culture medium. Following 5 passages, it was possible to plate out the virus and to isolate 6 single plaques on monkey kidney monolayers. Progenies of these plaques were further purified through 5 passages and the resulting material tested for its ability to grow in developing chick embryo. Of 5 plaques, 3 yielded progenies which multiplied without any difficulty in the egg. One failed to do so and the

TABLE 34 FATE OF MONKEYS INJECTED WITH SM-45 STRAIN OF ATTENUATED TYPE 1 VIRUS

		Intracerebral		Intraspinal	
Material	Days after Ingestion	TCD_{50} Inoc.	Ratio	TCD_{50} Inoc.	Lesions
Inoculum		5 5 5 2	0/5 0/5	4 5	0/5
Feces*	1–27†	9	0/4 0/4 0/4	3 2 2 2 1 2	3
4th T C	1–27†		0/5	7 2	

* Baby 39 days old at the ti on.
† Total fecal irus output

TABLE 35 [illegible] PLAQUE LINE. STOOL (FOX 1149) OF 1 YEAR-OLD ASYMPTOMATIC CHILD—I C 0/3*

Plq 15 a
I C 1/3

Plq 15 b
I C 0/3
↓ + MK
I C 1/4 [0]
I S 1/4 [1]

Plq 15 c
I C 1/3

Plq 15 d (7 2)†
I C 0/3
↓ + MK
I C 0/4
I S 2/4
I M 0/1
↓
Plq 25 (8 0)
I C 0/4 [1]
I S 0/4 [1]
↓ + MK (Pool 5) (7 0)

	I C	I S
Und	1/10 [1]	1/4
10	0/10 [1]	1/4 [1]
10	0/4 [0]	0/4 [0]
10 ³	0/4 [0]	0/4 [0]

↓
Pl 34 c (6 7)
I C 0/3 [0]
I S 1/5 [1]
↓ + MK (Pool 6) (6 5)
Und I C 0/6 [0]
10 I C 0/6 [0]
↓ + MK (Pool 7) (7 ?)

	I C	I S
Und	0/14 [0]	0/4 [0]
10	0/4 [0]	0/4 [0]
10	1/4 [1]	0/4 [0]
10	0/4 [0]	—

↓
Plq Pool 11 (6 7)
I C 0/4 [1]
I S 0/4 [0]

Plq 15 e
I C 2/3

Plq 16
I C 0/3

Pl 34 a
I C 0/5 [0/3]

Pl 34 b
I C 1/4 [1]

Pl 36
I C 0/4 [0/2]
I S ?/5 [2]

Plq Pool 8 (7 5)
I C 0/4 [1]
I S 1/4 [1]

Plq Pool 9 (6 5)
I C 0/4 [0]
I S 0/4 [0]

Plq Pool 10 (7 5)
I C 0/4 [0]
I S 0/4 [1]

Plq Pool 12 (7 7)
I C 0/4 [0]
↓ Cortone
I C & I S + I M 1/5 [1]
I S 0/5 [0]

Plq Pool 13 (7 5)
I C 0/4 [0]
I S 0/4 [0]

Plq = plaque
* Ratio of monkeys showing clinical signs after I C (intracerebral) I S (intraspinal) or I M (intramuscular) inoculation.
† Figures in ( ) = titers of virus in monkey kidney tissue culture all logs to base 10 MK = Monkey kidney tissue culture
‡ Figures in [ ] indicate number of monkeys in which histopathological examination revealed CNS lesions e g [0] adjacent to 0/4 indicates that all 4 monkeys were examined for histological lesions and none were found. Und = undiluted. Min = minimal
[illegible] of less than [0/3] adjacent to 0/4 indicate that only 3 out of the 4 were examined

TABLE 36 FATE OF MONKEYS INJECTED WITH ATTENUATED FOX 3 STRAIN FOLLOWING PASSAGE THROUGH HUMAN INTESTINAL TRACT*

MATERIAL	DAYS AFTER INGESTION	INTRACEREBRAL			INTRASPINAL		
		TCD_{50} INOC	PARALYTIC RATIO	LESIONS	TCD_{50} INOC	PARALYTIC RATIO	LESIONS
Feces	3–9	2 4	0/4	0/4	1 7	0/4	1/4?
5th T C	3–17 31–48	7 2	0/4		6 5	0/4	
		6 2	0/4		5 5	0/4	
					4 5	0/4	
					3 5	0/4	
					2 5	0/4	
	3–52†	7 2	0/4		6 5	0/4	

Baby 78 days of age at the time of irus adm ni tration
† Total fecal viru output concentr ted and us d a seed for tissue culture passages

TABLE 37 *COMPARATIVE PATHOGENICITY FOR CYNOMOLGUS MONKEYS OF 2 ATTENUATED STRAINS* OF TYPE 1 POLIO VIRUS

ROUTE	STRAIN	LOG OF TCD_{50} INOC	NUMBER OF MONKEYS		
			INOCULATED	PARALYZED	WITH SPECIFIC CNS LESIONS
Intracerebral	CHAT	7 2	4	0	0
		6 2	4	0	0
		5 2	4	0	0
		4 2	4	0	0
	N 90	5 0	4	0	1
		4 0	4	0	0
		3 0	4	1	3*
		2 0	4	0	2
		1 0	4	0	0
Intraspinal	CHAT	6 5	4	1	0
		5 5	4	1	2*
		4 5	4	0	0
		3 5	4	0	0
	N 90		3		2
			3		1
			3		1†
			3		0

Min mal loss of anterior horn cells t the cer
† Another m nkey showed traumatic type of les

results with 1 strain were equivocal The problems concerning this mixed virus population in relation to its characteristics in egg growths as well as other characteristics of the virus after passage through the infant alimentary tracts are being currently investigated

The word attenuation implies that an agent has undergone changes in its characteristics for instance loss of pathogenic properties for the original host However if an attenuated virus is to be used as a vaccine it must retain the immunogenic power of the original strain This has not always been the case Indiscriminate passages of the 17D strain of yellow fever virus through numerous tissue-culture generations led to the development of a strain which although infectious for mice had lost its immunogenic power for man Are we facing a similar problem *with the attenuation of polio viruses? The answer may well be in the affirmative*

Table 37 summarizes results of comparative titrations in monkeys of 2 attenuated sublines of the same Type 1 virus the SM strain Analysis of the results indicates that the N 90 is more pathogenic for monkeys than the CHAT strain Paralysis occurred in 1 monkey after intracerebral *inoculation and specific CNS lesions were* observed in monkeys inoculated both intraspinally and intracerebrally with low concentrations of the N 90 virus in contrast with the results obtained with the CHAT strain of lesser neurotropism in monkeys

The results of ingestion of these 2 strains of *virus by a group of infants is shown in Table* 38 These were groups of infants who had congenitally acquired passive antibodies and the concentration of antibodies was similar in the two groups who were approximately the same age The 2 strains were administered orally the less attenuated N 90 strain and the more attenuated CHAT *strain The less attenuated N 90* subline proved to be an excellent antigen Seven infants ingested $10^{\ 5}$ to $10^{3\ 5}$ tissue-culture doses of virus and of those 7 only 3 failed to develop active immunity Higher concentrations of this virus immunized every subject In marked contrast the seemingly more attenuated CHAT strain *failed to immunize 5 out of 5 infants who* received $10^{1\ 6}$ to $10^{4\ 5}$ tissue-culture doses of the virus and 1 out of 3 infants failed to develop active antibodies following ingestion of $10^{4\ 5}$ to $10^{\ 5}$ tissue-culture doses Only when given in

TABLE 38 COMPARATIVE ANTIGENICITY OF 2 STRAINS OF TYPE 1 POLIO VIRUS AT DIFFERENT STAGES OF ATTENUATION ADMINISTRATION TO INFANTS LESS THAN 6 MONTHS OLD

AMOUNT OF VIRUS FED IN TCD_{50} (RANGE)	RATIO† OF INFANTS FAILING TO REACT AFTER FEEDING VIRUS STRAIN	
	N 90	CHAT
2 5 3 5	‡3/7	NT
3 6–4 5	0/7	5/5
4 5–5 5	0/9	1/3
> 5 5	NT	0/5

E pres e l l g t b e of 10
† Num r t r = n b r f nf t f l ng to respon l den m to = mber of nf t ecei ing iru
‡ All 3 i ed 10 TCD se nd adm n u t n f 10 TCD_{50} resulted in n m n l g e po e

excess of 10 ^{5} tissue-culture doses did the *CHAT strain seem to elicit uniform antibody* response

There were 2 logs difference in immunogenic capacity between the more attenuated strain and the less attenuated strain of the same subtype You need 10 to $10^{3\ 5}$ tissue-culture doses to cause immunity after the original strain and you *need 10* ^{6} *tissue-culture dose of the more atten*uated to provoke immunogenic response

Granted that this series is small and the results may be characteristic only for this particular series nonetheless it is conceivable that it may represent a general trend in the field of attenuation of polio viruses This ultimately may *put the advocates of superattenuation in an* untenable position of recommending administration of gallons of polio virus to immunize 1 child and the creation of new criteria of attenuation may also have to become less exuberant in light of experimental facts In this frantic search for apathogenicity one should not forget that a *balance between modification of characteristics* and retention of antigenic properties has to be maintained in order to bring the work to its logical fruition

It is now more than 7 years since the TN strain was administered for the first time to a human subject with development of homotypic *antibodies following its ingestion Data sum*marized in Table 39 show persistence of antibodies in sera of 9 children who were fed the virus 6 years ago In 5 subjects there was no difference in antibody levels between specimens

TABLE 39 PERSISTENCE OF ANTIBODIES AGAINST TYPE 2 POLIO VIRUS IN SERA OF INDIVIDUALS FED TN STRAIN

Individual Number	Minimum Protective Titer* of Serum Obtained Months After Administration of Virus	
	1 11 Mos	60–72 Mos
2	2 0 (11)†	2 0 (72)
3	1 6 (2)	2 0 (72)
9	2 2 (3)	2 0 (72)
5	1 9 (1)	1 2 (72)
7	2 4 (1)	2 4 (72)
8	2 4 (1)	2 5 (72)
13	1 6 (1)	1 2 (60)
14	1 8 (13)	1 2 (72)
10	2 4 (1)	1 8 (60)

Log to base 10
† Number in ( ) = month after feeding

collected 1 to 3 months after virus ingestion and 6 years later In 4 cases a slight decrease was observed which is probably insignificant insofar as insusceptibility to alimentary infection is concerned

The homotypic antibody levels observed after ingestion of attenuated Type 1 virus shown in Table 40 have been maintained with minor fluctuations throughout almost a 3 year observation period

TABLE 40 PERSISTENCE OF ANTIBODIES AGAINST TYPE 1 POLIO VIRUS IN SERA OF INDIVIDUALS FED SM STRAIN

Individual Number	Minimum Protective Titer* of Serum Obtained Months After Administration of Virus		
	1 Mo	2–14 Mos	15–34 Mos
GE	2 4 (1)†	2 9 (9)	3 0 (28)
GO	1 5 (1)		1 8 (?7)
W	2 1 (1)	2 8 (9)	2 5 (?8)
BR	1 5 (1)	1 8 (12)	2 2 (26)
T	1 8 (1)	1 2 (12)	1 2 (31)
M	2 2 (1)	1 2 (14)	2.4 (15)
BO	2 5 (1)	1 8 (12)	1 8 (31)
SC	1 0 (1)	2 5 (2)	2 4 (33)
JC	1 5 (1)		2 5 (34)

Log to base 10
† Number in ( ) = month after feeding

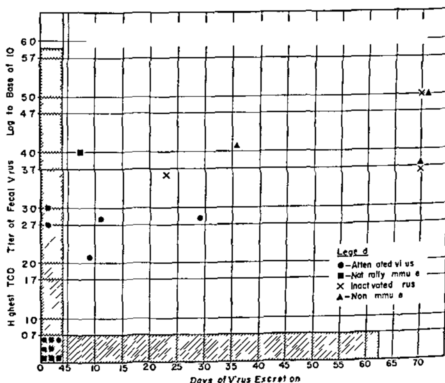


FIG 70 Duration of alimentary infection and concentration of fecal virus excreted by children infected orally with 10 TCD 0 of Type 1 virus

TABLE 41 RESISTANCE OF INTESTINAL TRACT OF CHILDREN TO INFECTION WITH 10 TCD_{50} OF TYPE I VIRUS IN NONIMMUNE NATURALLY IMMUNE AND IN CHILDREN VACCINATED WITH ATTENUATED VIRUS* AND WITH INACTIVATED VIRUS†

PREVIOUS HISTORY	ALIMENTARY INFECTION				SERUM ANTIBODY TITER	
	SUBJECTS EXCRETING VIRUS		DURATION CARRIER STATE	HIGHEST TCD_{50} TITER OF FECAL VIRUS	BEFORE INGESTION OF VIRUS	AFTER INGESTION OF VIRUS
	LESS THAN 4 DAYS	MORE THAN 4 DAYS	DAYS			
Attenuated Virus*	7				1 11 1 16 1 16 1 16 1 23 1 712 1 1 024	1 256 1 1 024 1 16 1 11 1 512 1 256 1 362
		3	8 11 28	10^{3}	1 4 1 16 1 23	1 712 1 256 1 256
Natural Immunity	3				1 64 1 256 1 16	1 64 1 178 1 16
		1	6	10	1 44	1 64
Inactivated Virus†	0					
		3	21 89 97	10	1 4 <1 2 1 64	1 1 024 1 256 1 256
Nonvaccinated	0					
		3	36 61 98	$10^{4.5}$	<1 2 <1 2 <1 2	1 256 1 256 1 91

* Vaccinated 2½ years before test.
† Vaccinated 6 months before test

We have also subjected several infants and children who were fed the virus to a refeeding of the same virus and these studies are summarized in Table 41 Note that 7 out of 10 subjects who had had a single feeding of attenuated Type I virus still had intestinal tract protection 2½ years later against a massive dose of the same virus given orally In the remaining 3 subjects re-exposure to the same agent resulted in sporadic excretion of the virus which was still recoverable 28 days after ingestion in 1 single case The highest concentration of the fecal virus never exceeded 10^{3} tissue-culture doses Booster effect on antibody titers was observed in 6 out of 10 subjects Antibody content in sera of 2 other subjects whose pre-exposure titers were high appeared to show a decrease but this may well be within the limits of experimental error of the test

Resistance of the alimentary tract to infection was observed also in 4 subjects who were found to be naturally immune before exposure to Type I virus Three did not excrete the virus and 1 did during 6 days concentration of the fecal

virus on one occasion reaching $10^{4\ 0}$ No booster effect was noted in any of the 4 subjects

In marked contrast to these results subjects who had been vaccinated with inactivated virus or were nonimmune developed an alimentary infection which lasted from 1 to 3 months with fecal virus titers surpassing by more than 2 logs those observed in the isolated instances of alimentary infection after re-exposure in the group originally immunized with living virus Rise in antibody was considerable in both the nonimmune group and in those vaccinated with inactivated virus (Fig 70) Although these results were obtained with a relatively small group of subjects the evidence is quite overwhelming in favor of prevention of alimentary carrier state through ingestion of living virus rather than through injection of inactive virus Apparently there are two mechanisms of defense against poliomyelitis—one central probably operating in the presence of circulating antibodies and the other local operating in the intestinal tract and not related to the level of circulating antibodies Inactivated virus may evoke the former the living virus may elicit both

In considering biologic and medical problems balance of values and sense of proportion should be carefully matched Protection of man against a disease is obtained at a price nothing in nature is given free and all efforts should be made to reduce the cost of this payment Yet in recent years considerable hue and cry has been raised that the attenuated living polio viruses with all their numerous advantages should fulfill criteria of perfection which have never been met before and never will be in other and similar fields There is a desire to consider any evidence of mutation almost in the light of a crime committed by the virus against nature However is it possible to consider that the only virus particles which will never mutate are those which do not exist?

The striving for a virus strain completely devoid of any neurotropic properties may lead to an agent which retains infectivity on experimental assay but is no longer infectious or antigenic for man In terms of such logic the strain should not cause alimentary infection and yet be able without multiplication to immunize the subject against disease by a mechanism not apparent in any other infection The advocates of safety do not want to pay any price for immunization yet exactly what are the costs one might have to pay for a method of immunization which would not only protect the vaccinated subject against the disease but also may lead to elimination of poliomyelitis?

From all the data accumulated to date with recently studied attenuated strains in 2 or 3 different laboratories there is no evidence that the degree of mutation of these polio viruses in the human intestinal tract or in any other susceptible system is such that it will ever lead to production of a strain which will be highly paralytogenic for man Experimental evidence supporting such ideas should be furnished by those who propound them not by us But let us suppose that an attenuated polio virus spreads in a community and in course of passages changes to a more virulent virus albeit there are no experimental and clinical data supporting such an idea In endemic areas covering most parts of the world such a virus is no greater a menace to the community than the numerous other strains which are isolated from feces of completely healthy children and more often than not are highly virulent for intraneurally injected monkeys Of the hundreds of nonimmune individuals who received attenuated polio viruses developed in the 2 laboratories none showed signs of illness 6 serial passages through the human intestinal tract have not led to a virus which is pathogenic for man or highly neurotropic for primates Thus there is every indication that if an attenuated polio virus were disseminated through a community unlikely as that may be it would play a prominent role in the ultimate replacement of the more virulent wild strains. If there is concern over the personal safety of the thousandth or the millionth individual who is to swallow the attenuated virus then it could be administered in the presence of antibodies acquired congenitally or with a gamma globulin injection or following vaccination with inactivated virus

Such procedures should satisfy even the most adamant adherents of the safety first school Strains available today for large scale clinical trials may be as good as they probably ever will be Those of us who have worked in this field for years have to reach a point where we refuse to search indefinitely for strains which very often will fill imaginary criteria of attenuation and while doing so subjecting hundreds and thou

sands of monkeys and chimpanzees to inoculations observations and histologic examinations

The time has come when a careful and patient evaluation of the attenuated viruses as immunizing agents against poliomyelitis may lead those who have a sense of proportion to the conclusion that the price one has to pay today for the comfort of future generations is indeed negligible

BIBLIOGRAPHY

Dulbecco R and Vogt M J Exper Med 99 167 1954

Fox J P and Penna H A Am J Hyg 38 152 1934

Koprowski H *In* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 128 133 New York New York Acad Sc 1957

——— World Health Organization Monograph No 26 p 335 New York Columbia 1955

Koprowski H Jervis G A and Norton T W Am J Hyg 55 108 1952

——— Proc Soc Exper Biol & Med 86 244 1954

Soper F L Smith H and Penna H Proc 3rd Internat Cong Microbiol p 351 Springfield Ill Thomas 1939

[illegible] and [illegible] of Orally Administered [illegible] Virus Vaccine

[illegible] ALBERT B SABIN

[illegible] determine [illegible] ed [illegible] production [illegible] eradication [illegible] The ca[illegible] harmless, im[illegible] tract is [illegible] The [illegible] have [illegible] can [illegible] antibody [illegible] antibody and [illegible] from Salk [illegible] ed strains has been found to reproduce the natural infectious process in the alimentary tract, there may be reason for expecting that the resulting immunity may also be as long-lasting but only the passage of time can establish whether or not such expectation is justified

Recent studies summarized in Table 4[?] have also shown that a single infection by attenuated strains can reproduce the resistance of the alimentary tract to subsequent infection that is found in naturally immune individuals but not in those immunized by the intramuscular injection of killed virus vaccin Tests on 4 different groups are shown One hundred thousand tissue-culture doses of the indicated type of attenuated virus were fed to the number of individuals in

TABLE 4 [illegible] ALIMENTARY TRACT TO INFECTION WITH ABOUT 10 PFU OF INDICATED ATTENUATED STRAINS [illegible] NATURALLY IMMUNE ADULTS AS WELL AS [illegible] RECEIVED SINGLE FEEDINGS OF ATTENUATED VIRUS OR 2 DOSES OF SALK VACCINE INTRAMUSCULARLY

CATEGORY	TYPE 1 LSc	TYPE 2 P 712	TYPE 3 LEON
No homotypic [illegible] high-avidity antibody	17/19*	15/15	17/17
Naturally acquired [illegible] antibody only (pH test) (with few exceptions may be result of heterotypic infection)	15/19	2/3	7/7
Naturally acquired [illegible] and high avidity antibody present	1†/11	2+1?/11‡	6/1?§
[illegible] 8 to 15 months before test	1*/10	1 /7	1?/6
No homotypic [illegible] or high-avidity antibody before Salk vaccine Received 2 doses Salk vaccine—tested 2 weeks to 3½ months after 2nd dose	9/9	6/7	9/9

* 17/19 = 17 of 19 individuals [illegible] excreted virus.
† This individual had a [illegible] Type 1 antibody [illegible] tests (1 3 high avidity and 1 8 low avidity) which might represent a [illegible] to infection [illegible] 3 polioviruses When he was fed Type 1 virus a second time 8 mo[illegible] was no multi[illegible] alimentary tract
‡ = only a trace of virus [illegible]
§ Note that in naturally imm[illegible] resistance of t[illegible] to [illegible] infection is highest for Type 1 and [illegible] for Type 3 [illegible] to Type [illegible] ingested Type 3 attenuated virus perhaps may be [illegible] that the sa[illegible] only a few [illegible] feeding 8 [illegible] affected by [illegible] days and [illegible] alimentary tract [illegible] multiplied [illegible] 15 mo[illegible] virus and [illegible] the [illegible]

dicated in the denominator and the number who excreted virus even for the briefest period are shown in the numerator No boost in antibody was detected in any person who failed to excrete virus In the first group without homotypic antibody all excreted virus except 3 adults who were fed the Type 1 virus strain There is reason to believe that these 3 individuals may have had a previous Type 1 infection which immunized their alimentary tract but left insufficient circulating antibody to be detected by the usual tests This is based on experimental observations

In the naturally immune group the majority exhibited complete resistance and the few who excreted virus generally did so for only brief periods and in reduced amounts The incidence of intestinal resistance was greater for Type 1 than for Type 3 virus In the group immunized by ingestion of attenuated strains 8 to 15 months earlier intestinal resistance was greatest the slight break throughs occurring in only 3 individuals who initially experienced only a limited intestinal infection or had a simultaneous infection with more than 1 type of polio virus In the volunteers whose antibody was induced by formalinized vaccine virus excretion was entirely comparable both in quantity and duration with that found in the first group without antibody There is therefore some reason for assuming that an orally administered attenuated polio virus vaccine would interfere with the implantation and the dissemination of the naturally occurring virulent strains

The real issue therefore concerns the safety of an attenuated vaccine not only as regards the viruses that are originally ingested but also of those that are excreted after varying periods of multiplication in the alimentary tract If there were strains of polio virus completely lacking in neurotropism for the most susceptible primate neurons regardless of dose and mode of inoculation and if such strains were otherwise harmless and unlike other living organisms did not mutate there would be little diffi ulty in reaching a quick decision regarding their use on a large scale Since the extensive studies of the past 4 years have failed to reveal such polio viruses the remainder of this paper will be devoted to a summary of our current knowledge about the signifi ance of various degrees of neurotropism as it is measured quantitatively in monkeys and of the variations in neurotropic activity of different strains before and after propagation in the alimentary tra t

The spectrum of currently available strains shown in Table 43 ranges from the most neurotropic which paralyze monkeys after intracerebral injection of 1 to 10 tissue-culture infective doses to the least neurotropic which are inactive except in a limited way in an occasional monkey after direct spinal inoculation of 1 000 000 or more tissue-culture infective doses Strains which may be regarded as being attenuated at least 10 millionfold because 10 000 000 or more tissue-culture infective doses are inactive in most intracerebrally inoculated monkeys may still be paralytogenic intraspinally in doses of 10 to 100 tissue-culture infective doses Thus the strains at the least neurotropic end of the spectrum represent an attenuation that is in excess of 100 millionfold but these strains still produce neuronal lesions of varying extent in a varying proportion of spinally inoculated monkeys which exhibit no paralysis However it has also become evident from tests on large numbers of chimpanzees that polio viruses that are only partly attenuated as determined by their intracerebral and spinal activity in monkeys are not paralytogenic in maximal doses by the spinal route in chimpanzees Some of these strains also pro luce no demonstrable lesions As regards chimpanzees one can speak of a completely non neurotropic polio virus Since th incidence of paralysis among chimpanzees that are fed highly virulent virus is approximately 70 per cent corresponding to the maximal paralytic attack rate recorded for human beings—I am referring to the virgin soil epidemic among highly inbred Eskimos—there is at least some reason for assuming that the most susceptible human neurons are comparable to those of chimpanzees These observations taken togeth r with the fact that viruses recovered from the nervous system of paralytic patients invariably exhibit high intracerebral paralytogenic activity in monkeys provide us with the only criteria that we now have for estimating the potential safety for human beings of viruses in a given range of the monkey neurotropic spectrum It is of course possil le that other fa tors exclusive of neurotropism such as a limited ability for multiplication in extraneural tissues outside the alimentary tract

TABLE 43 NEUROTROPIC SPECTRUM OF KNOWN POLIO VIRUSES IN RELATION TO DIFFERENT PRIMATE NEURONS

Most Neurotropic 1—10 TCID Paralysis	Cynomolgus Monkeys	Least Neurotropic 10^{6}—$10^{8\,8}$ TCID No Paralysis
Brainstem Neurons 10^{1} 10^{2} 10^{3} 10 10^{5} 10^{6} 10^{7}		Lumbar Cord Neurons 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7}
	Viruses active in this range in monkeys *Not paralytogenic in chimpanzees* in doses of 10^{6}—10^{7} TCID intraspinally	

may also influence the safety of orally adminis tered virus

In the very first tests in chimpanzees and hu man volunteers it was found that after feeding partially attenuated strains the virus excreted in the stools was more neurotropic for monkeys than that which was fed The results obtained with the stools of 7 volunteers at different times after ingestion of the least attenuated and most unstable Type 1 virus are shown in Table 44 What is shown here are the tests after ingestion of the *least* attenuated and most unstable Type 1 virus Although the original stools of only 2 in dividuals exhibited some paralytogenic activity in monkeys the presence of more neurotropic particles in most of the stool specimens becomes evident when the stool is cultured for only 1 pas sage—and I must stress that Too many passages give a change in the virus population This virus had only 1 passage and larger amounts were inoculated intracerebrally That the more neuro tropic particles do not necessarily overgrow the others is evident in the tests on volunteer Mal whose 9-day stool exhibited increased neuro-tropic activity while that obtained at 56 days and 140 days—and this is the longest carrier state observed in our study—was inactive at the 100 000 TCID level The most active stool cul ture in this series that of volunteer Har failed to produce paralysis in 3 chimpanzees inoculated intraspinally with more than 1 000 000 tissue culture infective doses of virus Similar negative results with 2 other strains were obtained in 6 chimpanzees inoculated with stool cultures of similar intracerebral neurotropic activity in mon keys Thus there is at least some reason for as suming that the increase in monkey neurotrop-ism observed in these tests is not necessarily in the harmful range for human beings Although this particular Type 1 strain was unstable even on propagation in vitro similar although less frequent and less extensive increases in neuro-tropism were observed with 4 naturally occur ring attenuated Type 1 strains This is quite a general property It also became evident that the extent of increase in neurotropism after multi plication in the alimentary tract might be re lated to the degree of attenuation of the virus that was fed The results of tests on early and late stools of 5 volunteers who were fed the highly attenuated Type 1 LSc strain (Table 45) yielded no paralysis in any of the 42 monkeys inoculated intracerebrally with amounts of virus up to 800 000 tissue-culture infective doses The reason some of the original stools were not tested is that they contained too little virus to be of significance The stools of 3 chimpanzees that ingested large amounts of this strain yielded similar results not only in intracerebrally but also in spinally inoculated monkeys However the results of spinal tests in monkeys with the stool cultures of 6 volunteers indicated an in crease in neurotropic activity in this higher range of the spectrum in at least 3 individuals Nevertheless these were the best results obtained with any of the Type 1 strains tested and there fore this strain was selected for further study

Essentially similar results were obtained with the Type 2 and the Type 3 polio viruses and the strains selected for further study exhibited the least neurotropism after multiplication in the alimentary tract

Since all the strains tested up to this point had been purified only by the terminal dilution

TABLE 44 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF VIRUS EXCRETED IN STOOLS OF HUMAN VOLUNTEERS FED LEAST ATTENUATED AND MOST UNSTABLE TYPE 1 VIRUS (MAHONEY KP 33)

Volunteer	Days After Ingestion of Virus	Original Stool: TCD_{50} Inoculated Log 10	Original Stool: Result	Stool Culture: TCD_{50} Inoculated Log 10	Stool Culture: Result
Wal	6	3 2	0 0	5 2	0 0 11
Hac	7	4 2	0 0	5 9	0 8 9
Ric	10	3 2	0 0	5 7	0 9 9
Har	10	4 2	0 8 23	4 9	8 8 11
Car	14	2 5	0 0	4 9	0 7 10
Tro	14	5 0	0 0 0	4 9	0 0 0
Tro	28	3 2	0 0 0	5 9	0 8 9
Mal	9	3 2	0 0 15	5 4	0 8 16
Mal	56	3 2	0 0 0	5 [illegible]	0 0 0
Mal	140	—	—	4 9	0 0 0 0
Total		2 5–5 0	3/23	4 9–5 9	14/31

11 = in b tion period (d ys) f p alyzed m nk y 0 = no p lys

Note Cult fl d fed t lunt e w ot par lyt gen c f m nk y inocul ted int erebrally with m ll o l rg d but sub equ t pas ge p ep ed f om it u lly paraly ed a y g pr port n Intra p lly th m ll t d s were p alyt g n f r cyn m lgu m key b t ne th l rg r mall d w r par lyt g n n h mp

TABLE 45 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF VIRUS EXCRETED IN STOOLS OF HUMAN VOLUNTEERS FED MOST HIGHLY ATTENUATED TYPE 1 VIRUS PURIFIED ONLY BY TERMINAL DILUTION TECHNIC (LSc)

Volunteer	Days After Ingestion of Virus	Original Stool: TCD_{50} Inoculated Log 10	Original Stool: Result	Stool Culture: TCD_{50} Inoculated Log 10	Stool Culture: Result
Moo	7	3 2	0 0 0	4 9	0 0 0
Moo	11	—	—	5 9	0 0 0
Kut	7	3 2	0 0 0	4 7	0 0 0
Kut	21			5 9	0 0 0
Mar	7	3 2	0 0 0	4 7	0 0 0
Mar	21			4 2	0 0 0
Mic	7	2 5	0 0 0	5 2	0 0 0
Mic	14			5 7	0 0 0
Ree	7			5 2	0 0 0
Ree	14			5 2	0 0 0
Total		2 5–3 2	0/12	4 2–5 9	0/30

Note C lt r flu l f l and l equ nt passages fro it in r l lv i t in nt acer l rally inoculated monk ys g en t l d

TABLE 46 INTRACEREBRAL ACTIVITY IN CYNOMOLGUS MONKEYS OF OPTIMUM SINGLE PLAQUE STRAINS OF EACH TYPE OF POLIO VIRUS GROWN IN 20 TO 25 LITER LOTS OF MONKEY KIDNEY TISSUE CULTURE

TYPE	STRAIN	PFU VIRUS PER ML	PARALYTOGENIC EFFECT OF 1 ML OF INDICATED DILUTION	
			UNDILUTED	10^{-1}
1	LSc 2 ab	4 2 x 10^7	0/10	0/5
2	P 712 Ch 2 ab	3 6 x 10^7	0/10	0/5
3	Leon 12 a b	4 3 x 10^7	0/10	0/5

Note Intracerebral inoculation of 10^7 PFU or more of other lots of these strains in groups of 10 monkeys also yielded negative results Intramuscular (deltoid) injection of about 10^8 PFU or more (2 ml undiluted culture fluid) of each strain in groups of 10 to 15 cynomolgus monkeys was also without paralytogenic effect

technic in culture tubes and may have represented progeny originally derived from more than 1 virus particle it was possible that any increase in neurotropic activity of virus excreted in the stools may have been due to the plating out in the alimentary tract of a pre-existing mixed population of virus particles in the culture fluid that was fed When the populations of the 3 optimum strains were dissected so to speak by obtaining the progeny of many single triply purified plaques it was indeed found by quantitative spinal tests in monkeys that the virus particles making up each strain were not homogeneous in their neurotropic activity some exhibited approximately the same spinal activity as the total population making up the original strain others were distinctly more neurotropic and 1 or 2 among 9 or 10 particles tested from each strain exhibited the least neurotropic activity that had been encountered thus far Since repeated *in vitro* subcultures of these highly attenuated single plaque derivatives yielded similar results on spinal inoculation in monkeys it became possible to test in a definitive way the difference in the viral populations produced by many cycles of multiplication in the alimentary tract from those produced during the relatively few cycles of multiplication in tissue cultures in vitro

The progeny of 37 separate triply purified plaques derived from 6 different Type 1 strains were tested by spinal inoculation in monkeys before selecting the optimum Type 1 strain Although a few other plaques yielded virus that was as highly attenuated none was superior to it The Type 2 single plaque strain that was ultimately selected was derived from a stool culture of a chimpanzee that had been fed the optimum single plaque virus segregated from the P 712 strain—a naturally occurring strain The Type 3 strain is derived from the optimum plaque segregated from the attenuated Leon virus Approximately 25 liter lots of each of these strains then were grown in rhesus kidney cultures with the generous co-operation of the Merck Sharp and Dohme Research Laboratories After Seitz filtration and appropriate tests in monkeys chimpanzees rabbits guinea pigs mice and tissue cultures aliquots of these lots were fed to 105 adult volunteers The standard dose, based on previous observations in human beings was 0 01 ml of culture fluid — approximately 100 000 plaque forming units — and this was given in a teaspoonful of cherry syrup Many stool specimens from each of these individuals were tested in tissue culture and no agents other than the ingested polio viruses were recovered

In order to be able to evaluate the significance of the tests for neurotropic activity of the virus found at different times in the stools of these volunteers it is necessary to examine the data on the viruses that were fed

Table 46 shows the tests for intracerebral and intraspinal activity in cynomolgus monkeys of the large lots of each type of virus used in these tests Completely negative results were obtained in these as well as in preceding intracerebral tests even when as much as 40 000 000 plaque forming units were inoculated in large numbers of monkeys The spinal tests show that only an occasional monkey exhibited a slight localized or questionable effect after inoculation of very

TABLE 47 INTRACEREBRAL PARALYTOGENIC ACTIVITY IN MONKEYS OF VIRUS CULTURED FROM STOOLS OF VOLUNTEERS FED OPTIMUM TYPE 1 SINGLE PLAQUE STRAIN (LSc 2 AB)

STOOL CULTURES FROM INDICATED NUMBER OF VOLUNTEERS OR INDIVIDUAL VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	TCD₅₀ INOCULATED LOG 10	RESULT
20	6—7	6 7—7 9	1 /80
2	14	7 0—7 4	0/8
4	21	7 0—7 4	1/16
Bli	28	6 9	0/4
Hou	28	7 7	1/4
		6 7	1/4
		5 7	0/4

The monkey exhibited paralysis of one arm 22 days after inoculation but despite the presence of typical lesions no cytopathogenic virus was recovered from the spinal cord

Stool culture from each volunteer inoculated in 4 [illegible] monkeys

large doses Doses in the range of 100 000 000 plaque forming units inoculated in the deltoid muscles of large numbers of cynomolgus monkeys were also without effect

Table 47 shows the results of intracerebral monkey tests on the stool cultures of 20 volunteers at 6 to 7 days after ingestion and on 8 stool cultures obtained at 14 to 28 days the longest period of excretion in this group of volunteers It should be noted that virus multiplication in the alimentary tract begins within 24 to 48 hours after ingestion and that at 6 to 7 days the virus in the stools already represents the progeny of many cycles of multiplication Among the 80 monkeys inoculated with 5 000 000 to 80 000 000 TCD₅₀ of virus from the 6- to 7-day stool cultures only 1 exhibited slight paralysis at 22 days after inoculation and no cytopathogenic virus was found in its spinal cord However 2 of the 8 monkeys inoculated with 5 000 000 or 50 000 000 TCD₅₀ of the 28-day stool culture of 1 volunteer exhibited paralysis indicating that virus in this particular stool had a slightly greater neurotropism than the virus that was fed This interpretation is confirmed by the results of the spinal tests in monkeys shown in Table 48 The 28-day stool culture of volunteer Hou shows a distinct increase in spinal activity over that found in his 7-day stool culture and in the 14 to 28-day stool cultures of the other 7 volunteers tested

The same dose of this Type 1 virus was also fed to 5 children who were 5 to 11 years of age

TABLE 48 SPINAL ACTIVITY IN MONKEYS OF VIRUS CULTURED FROM STOOLS OF VOLUNTEERS FED OPTIMUM TYPE 1 SINGLE PLAQUE STRAIN (LSc 2 AB)

VOLUNTEER	DAYS AFTER INGESTION OF VIRUS	TCD INOCULATED LOG 10	RESULT
Hou	7	5 4	1/4
	28	6 0	4/4
		5 0	2/4
		4 0	3/4
Bli	7	5 4	2/4
	28	5 2	1/4
Bu	14	5 7	0/4
Mil	14	5 7	1/4
Cit	21	5 7	0/4
Cris	21	5 7	0/3
Koc	21	5 7	2/4
Mar	21	6 0	2/3

and possessed no antibody for any of the 3 types of polio virus Two stool cultures from each child obtained 5 to 10 and 17 to 21 days after ingestion of virus were tested in a total of 40 intracerebrally inoculated monkeys with results similar to those shown for adults in Table 47 Here also the spinal tests revealed an increase in neurotropic activity

Table 49 shows the results of intracerebral tests in monkeys with 20 stool cultures One cannot get anywhere by testing a few stools of a few individuals Of course if one has a partly attenuated strain it is satisfactory But with

ministration the response is so fast even in those without previous exposure that there seems to be no difference However, a recent boost in antibody by formalinized vaccine has been found to interfere with the boosting capacity of an alimentary infection Dr Salk aided us in some of this work We carried out the tests together Similarly a dose of killed virus vaccine may be without effect when it is administered intramuscularly at too short an interval following a previous dose When live virus is given by mouth to individuals who previously had killed virus vaccine an interval of at least 3 months would appear to be desirable

No strains that can multiply elsewhere in the body but not in the alimentary tract have yet been found In fact my earliest studies have shown the reverse namely that intramuscular injection of an attenuated strain may be ineffective except when it localizes in the alimentary tract While the results of the present study clearly indicated that relatively more neurotropic mutants can occasionally arise in the alimentary tract they also established that when highly attenuated strains are fed the resulting mutants are still in that attenuated range of the neurotropic spectrum in which a million or more tissue-culture infective doses are harmless even after direct inoculation in the spinal cord of chimpanzees Therefore they may be expected to be harmless for man

Our own previous studies in 133 volunteers and the tests of Koprowski and of Dick on many hundreds of individuals with strains of different degrees of attenuation as well as our present tests in 110 individuals with the very highly attenuated strains were all without any harmful effects However it would probably require tests on tens of thousands of people to establish whether the theoretical expectations of safety are confirmed in actual practice Simultaneous feeding of such a vaccine to entire families and communities during seasons of the year when polio viruses naturally disseminate poorly or in communities where polio viruses of varying degrees of virulence are known to be spreading extensively already might be the best way to conduct such tests But where and under what circumstances would tests on increasingly larger numbers of people be justified? The definite reduction in the incidence of paralytic poliomyelitis achieved thus far by killed virus vaccine may naturally incline to a decision that in countries where mass application of killed virus vaccine is feasible it be given an opportunity to show what can be achieved over a period of years If the passage of time should prove that immunity resulting from the killed virus vaccine supplemented in at least some individuals by natural infection is indeed long lasting there might never be any need for considering the use of a live virus vaccine If however time should prove that the immunity conferred by a killed virus vaccine is of relatively short duration in a large proportion of individuals then consideration might be given to supplementation of the waning immunity by feeding of the best available attenuated vaccine a procedure that may be expected not only to reinforce the humoral immunity but also to induce that resistance of the intestinal tract to reinfection which could provide the only means for the possible eradication of poliomyelitis

On the other hand in countries where mass application of a killed virus vaccine is not feasible and where polio viruses of varying degrees of virulence are already known to be undergoing extensive spread in the population there would appear to be sufficient justification for initiating at this time trials of the currently available tested lots of highly attenuated polio virus vaccine And such trials are now actually in progress Finally I should like to say that it is obvious—at least so it seems to me—that more work time and experience ultimately will teach us the best way to eliminate poliomyelitis as a threat to human well being

BIBLIOGRAPHY

Dick G W A and Dane D S The evaluation of TN and SM virus vaccines Brit M J 1 70 1957

Koprowski H *et al* Immunization of infants with living attenuated poliomyelitis virus laboratory investigations of alimentary infection and antibody response in infants under 6 months of age with congenitally acquired antibodies J.A M A 162 1281 1956

Paul J R Horstmann D M Melnick J L Niederman J C and Deutsch J Immunization against poliomyelitis killed vaccine followed by induced infection with live virus *in* Cellular Biology Nucleic Acids and Viruses

(Special Publication) vol 5 pp 141 147 New York New York Acad Sc 1957

Ramos Alvarez M and Sabin A B Intestinal viral flora of healthy children demonstrable by monkey kidney tissue cultures Am J Pub Health 46 295 1956

Sabin A B Behavior of chimpanzee avirulent poliomyelitis viruses in experimentally infected human volunteers Am J M Sc 230 1 1955

——— Pathogenesis of poliomyelitis—reappraisal in the light of new data Science 123 1151 1956

——— Present status of attenuated live virus poliomyelitis vaccine J A M A 162 1589 1956

——— Properties of attenuated polioviruses and their behavior in human beings *in* Cellular Biology Nucleic Acids and Viruses (Special Publication) vol 5 pp 113 127 New York New York Acad S 1957

Epidemiology of Poliomyelitis in Populations Before and After Vaccination with Inactivated Viruses

DR JOHN P FOX*

Observations of the occurrence of poliomyelitis and more recently of past and current infection as distinct from disease have led to a reasonable hypothesis as to the epidemiology of poliomyelitis Although not all aspects are accepted universally a brief summary is an essential introduction to the work to be described This hypothesis should explain such phenomena as (1) the relatively recent emergence of poliomyelitis as an epidemic disease (2) the relative restriction of epidemic disease to the more highly developed portions of the temperate zones of the earth (3) the original characteristic age pattern and the shifts in that pattern toward older age groups (4) certain correlations between various socioeconomic factors and both infection and disease and (5) the relative infrequency with which infection results in paralytic disease

Etiologically we recognize a family of small hardy viral agents composed of at least 3 distinct but antigenically related members which apparently exist in nature only in relation to man While respiratory transmission may occur the oral portal of entry is usually postulated with consequent infection of the alimentary tract and reasonably prolonged shedding of the virus in the feces Variations in the age pattern of infections are explained best in terms of variations in the specific pathways predominantly followed by fecal virus in reaching the oral portal Utilization of the numerous possible indirect pathways i e polluted food and water and flies is related inversely to the level of environmental sanitation and probably predominates in underdeveloped areas In areas of adequate environmental sanitation personal contact among the usually susceptible and less hygienic young-child segment of the population is probably the dominant mechanism Significant neurologic disease as a consequence of infection is determined by the operation of numerous factors including strain virulence host age and such predisposing host factors as fatigue recent inoculations pregnancy and absence of tonsils Increasingly since about 1880 the originally tolerable balance between polio viruses and the human host has been disturbed probably chiefly by influences operating to delay infection to an age when it is apt to result in disease However it still remains true that relatively few infections result in significant disease

With the tremendous technical advances resulting from the adaption of tissue-culture methods to polio virus cultivation[6 4 9 30] prospective studies of the natural process of infection without reference to disease became feasible That to be described was initiated in southern Louisiana in 1953 with the hope that it would permit new insight into such diverse problems as defining the exact mechanisms of virus transmission and those factors which determine that a particular infection will result in paralytic disease the significance of postinfection immunity with reference to duration and influence on both homologous and heterologous reinfection and the phenomenon of the seasonal occurrence of disease The observations centered about a stable group of households recruited to be reasonably representative with respect to residence family size race and economic status Well before all of the questions posed had been answered it became necessary to offer the study group the benefits of artificial immunization With a primary 2-dose course of Salk vaccine completed during January 1956 the study was reoriented toward determining the influence of vaccine induced immunity on the natural occurrence of alimentary tract infection and viral dissemination In January 1957 the third or *booster dose* of vaccine was administered and the observations have continued with the same orientation

* Written in collaboration with Dr Henry M Gelfand Dr Dorothy R LeBlanc and Dr D ght n F R w

The purpose of the present paper is to review briefly the previously reported observations in the period 1953 to 1955 prior to vaccination [8 9 10 1] to present more completely the 1956 observations which have been reported only partially elsewhere[11] and to report briefly on observations made in the first few months of 1957 after the booster dose of vaccine

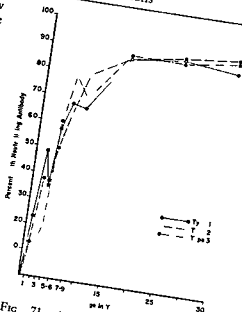


FIG 71 Age specific percentages of household associates of index children with neutralizing antibodies to Types 1 2 and 3 polio viruses when admitted to the study in 1953 (Fox J P *et al* Am J Hyg 65 344)

FIELD AND LABORATORY METHODS

The most important single facility was the study group itself This originally comprised 157 households recruited in 1953 having in common 2 essential attributes (1) a newborn child to serve as the known nonimmune index individual and (2) a high probability of co-operativeness continuing through several years of observation Otherwise they were divided as equally as possible into a number of subgroups defined on the basis of family size area of residence race and within the white group economic status Three study areas were established these consisted of urban New Orleans with a relatively low prior poliomyelitis morbidity (8 per 100 000 population in 1950 1954) urban Baton Rouge with a high prior morbidity (29 9) and 4 semirural parishes in south-central Louisiana of low past incidence (13 1) designated as the Evangeline Area As vaccination was offered in January 1956 there remained 136 households containing 139 original index children 675 older associates and 76 younger siblings With the re orientation of the study 18 families withdrew leaving 118 under observation after vaccination During 1956 an additional 8 families were lost. However 9 new families each closely related to an original study household were added so that the 1957 observations began with a total of 119 households

On admission of each household a basic record was opened which contained pertinent current and historical information Each household member was bled on admission to the study and annually thereafter in order to permit detection of possible episodes of infection which had not involved the index child At monthly intervals continuing through 1955 blood and stool specimens were collected from the index child and interval information was obtained concerning the entire household Following vaccination in 1956 routine bleedings were made only in relation to vaccination but stools were collected twice monthly from all children under 15 years of age In all cases detection of infection of any child led to a special visit for added information and for specimens from household and other indicated associates

The laboratory methods have been described in detail elsewhere [10] Although changes were made as improved methods were developed the basic observations were derived from the use of monolayer film cultures of trypsin-dispersed monkey kidney cells [3] This applies not only to virus isolations but also to tests for neutralizing antibody This latter point is of importance since according to Sabin [6] this method reveals only high avidity antibody With the exception of those specimens obtained from young children by heel or finger puncture which could not be tested in final dilution lower than 1 10 all statements as to absence of antibody are based on testing sera in final dilution of 1 2

PATTERNS OF SERO-IMMUNITY IN THE STUDY GROUP

Although the study group constitutes but a small sample of the total population and is repre

Epidemiology of Poliomyelitis in Populations Before and After Vaccination with Inactivated Viruses

DR JOHN P FOX*

Observations of the occurrence of poliomyelitis and more recently of past and current infection as distinct from disease have led to a reasonable hypothesis as to the epidemiology of poliomyelitis Although not all aspects are accepted universally a brief summary is an essential introduction to the work to be described This hypothesis should explain such phenomena as (1) the relatively recent emergence of poliomyelitis as an epidemic disease (2) the relative restriction of epidemic disease to the more highly developed portions of the temperate zones of the earth (3) the original characteristic age pattern and the shifts in that pattern toward older age groups (4) certain correlations between various socioeconomic factors and both infection and disease and (5) the relative infrequency with which infection results in paralytic disease

Etiologically we recognize a family of small hardy viral agents composed of at least 3 distinct but antigenically related members which apparently exist in nature only in relation to man While respiratory transmission may occur the oral portal of entry is usually postulated with consequent infection of the alimentary tract and reasonably prolonged shedding of the virus in the feces Variations in the age pattern of infections are explained best in terms of variations in *the specific pathways predominantly followed by* fecal virus in reaching the oral portal Utilization of the numerous possible indirect pathways i e polluted food and water and flies is related inversely to the level of environmental sanitation and probably predominates in underdeveloped areas In areas of adequate environmental sanitation personal contact among the usually susceptible and less hygienic young-child segment of the population is probably the dominant mechanism Significant neurologic disease as a consequence of infection is determined by the operation of numerous factors including strain virulence host age and such predisposing host factors as fatigue recent inoculations pregnancy *and absence of tonsils Increasingly since* about 1880 the originally tolerable balance between polio viruses and the human host has been disturbed probably chiefly by influences operating to delay infection to an age when it is apt to result in disease However it still remains true that relatively few infections result in significant disease

With the tremendous technical advances resulting from the adaption of tissue-culture methods to polio virus cultivation [6 12 27 30] prospective studies of the natural process of infection without reference to disease became feasible That to be described was initiated in southern Louisiana in 1953 with the hope that it would permit new insight into such diverse problems as defining the exact mechanisms of virus transmission and those factors which determine that a particular infection will result in paralytic disease the significance of postinfection immunity with reference to duration and influence on both homologous and heterologous reinfection and the phenomenon of the seasonal occurrence of disease The observations centered about a stable group of households recruited to be reasonably representative with respect to *residence family* size race and economic status Well before all of the questions posed had been answered it became necessary to offer the study group the benefits of *artificial immunization With a primary* 2-dose course of Salk vaccine completed during January 1956 the study was reoriented toward determining the influence of vaccine induced immunity *on the natural occurrence* of alimentary tract infection and viral dissemination In January 1957 the third or booster dose of vaccine was administered and the observations have *continued with the same orientation*

*Written in collaboration with Dr Henry M Gelfand Dr Dorothy R LeBlanc and Dr Dighton F Rowan

The purpose of the present paper is to review briefly the previously reported observations in the period 1953 to 1955 prior to vaccination[8 9 10 1] to present more completely the 1956 observations which have been reported only partially elsewhere[11] and to report briefly on observations made in the first few months of 1957 after the booster dose of vaccine

FIELD AND LABORATORY METHODS

The most important single facility was the study group itself This originally comprised 157 households recruited in 1953 having in common 2 essential attributes (1) a newborn child to serve as the known nonimmune index individual and (2) a high probability of co-operativeness continuing through several years of observation Otherwise they were divided as equally as possible into a number of subgroups defined on the basis of family size area of residence race and within the white group economic status Three study areas were established these consisted of urban New Orleans with a relatively low prior poliomyelitis morbidity (8 per 100 000 population in 1950 1954) urban Baton Rouge with a high prior morbidity (29 9) and 4 semirural parishes in south-central Louisiana of low past incidence (13 1) designated as the Evangeline Area As vaccination was offered in January 1956 there remained 136 households containing 139 original index children 675 older associates and 76 younger siblings With the reorientation of the study 18 families withdrew leaving 118 under observation after vaccination During 1956 an additional 8 families were lost However 9 new families each closely related to an original study household were added so that the 1957 observations began with a total of 119 households

On admission of each household a basic record was opened which contained pertinent current and historical information Each household member was bled on admission to the study and annually thereafter in order to permit detection of possible episodes of infection which had not involved the index child At monthly intervals continuing through 1955 blood and stool specimens were collected from the index child and interval information was obtained concerning the entire household Following vaccination in 1956 routine bleedings were made only in relation to vaccination but stools were collected

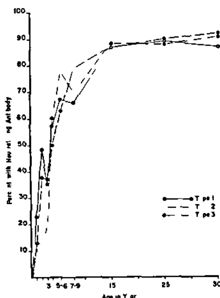


FIG 71 Age specific percentages of household associates of index children with neutralizing antibodies to Types 1 2 and 3 polio viruses when admitted to the study in 1953 (Fox J P *et al* Am J Hyg 65 344)

twice monthly from all children under 15 years of age In all cases detection of infection of any child led to a special visit for added information and for specimens from household and other indicated associates

The laboratory methods have been described in detail elsewhere[10] Although changes were made as improved methods were developed the basic observations were derived from the use of monolayer film cultures of trypsin-dispersed monkey kidney cells[3] This applies not only to virus isolations but also to tests for neutralizing antibody This latter point is of importance since according to Sabin[6] this method reveals only high avidity antibody With the exception of those specimens obtained from young children by heel or finger puncture which could not be tested in final dilution lower than 1 10 all statements as to absence of antibody are based on testing sera in final dilution of 1 7

PATTERNS OF SERO-IMMUNITY IN THE STUDY GROUP

Although the study group constitutes but a small sample of the total population and is repre

Table 56 Distribution of Episodes of Household Infection in Southern Louisiana by Month and by Virus Type in 1954 1955 Before Vaccination and in 1956 After Vaccination

Year	Virus Type	J	F	M	A	M	J	J	A	S	O	N	D	Total
		Number of Episodes in Month Indicated												
1954	1								2	4	1		1	8
	2						4	10	2		2	1	1	20
	3			1		3	3	9	2	3	7	1		29
	All			1		3	7	19	6	7	10	2	2	57
1955	1	2		1	4	2	2	6	6	1			1	25
	2	1		2		1	2	2	3	2		1	1	15
	3	2							3			2	1	8
	All	5		3	4	3	4	8	12	3		3	3	48
1956	1		2	2	2	4	4	3	5	2	5			29
	2				1	1	2							4
	3				1		5	5	3					14
	All		2	2	4	5	11	8	8	2	5			47
1954–1956	All	5	2	6	8	11	22	35	26	12	15	5	5	152

Table 57 Polio Virus Types Isolated from Reported Cases of Poliomyelitis and from Nonclinical Infections in Primary Index Children Southern Louisiana 1954 1955*

Type of Polio Virus	Isolations in 1954				Isolations in 1955				Total Isolations			
	Cases		Index Infections		Cases		Index Infections		Cases		Index Infections	
	No	%	No	%	No	%	No	%	No	%	No	%
1	6	35	8	14	24	50	25	52	30	46	33	31
2	3	18	20	35	4	8	15	31	7	11	35	33
3	8	47	29	51	20	42	8	17	28	43	37	35
Total	17	100	57	100	48	100	48	100	65	100	105	100

Gelfand H M *et al* Am J Hyg 65 367 1957

viruses and looking at the 1956 data perhaps of Type 2 virus as well However it should be pointed out that the distribution by type of viruses isolated from clinically manifest infections occurring in the study areas did not parallel that of the viruses isolated from the household episodes This is shown in Table 57 Type 1 virus in 1954 and Type 3 virus in 1955 were disproportionately common among the instances of disease In effect this suggests that *among* the more virulent strains Types 1 and 3 predominated in 1954 and 1955 respectively

Distribution of the infections in index children by their age in months revealed *no obvious* age of greater susceptibility The earliest infection was detected as of 23 days of age and a

TABLE 58 INFECTIONS AMONG ASSOCIATES OF INFECTED INDEX CHILDREN IN 1954 1955 PRIOR TO VACCINATION IN RELATION TO HOMOLOGOUS IMMUNITY STATUS PRIOR TO HOUSEHOLD INFECTION EPISODE

PRIOR IMMUNITY	STATUS IN RELATION TO INDEX CHILD	SEROLOGIC RESPONSE TO INFECTION	NUMBER OF PERSONS: TOTAL	NUMBER OF PERSONS: EXCRETING VIRUS	PROVED INFECTIONS: RATIO*	PROVED INFECTIONS: %
None	Adult	None Yes	4 11 (7)†	0 3	11 15	73
	Sibling	None Yes	8 125 (97)	0 54	125 133	94
	All Associates	None Yes	12 136 (104	0 57	136 148	92
Yes	Adult	None Yes	179 (115) 24 (16)	3 0	27 203	13
	Sibling	None Yes	64 (44) 37 (30)	1 3	38 101	38
	All Associates	None Yes	243 (152) 61 (46)	4 3	65 304	1

* Ratio of number of persons infected to number exposed

† Figures in parentheses indicate number of persons from whom the single stool specimen obtained was collected within ... of the first specimen during infection of the household

total of 20 occurred during the first 6 months of life 4 of these in the demonstrated presence of homologous maternally derived antibody Family size and race or economic status were of importance infections being more frequent in one or both of the years 1954 and 1955 among index children with 2 or more older siblings and among those in Negro or lower-economic white families In this connection it is interesting to note that Melnick and co-workers [21] in their prospective studies in Arizona and West Virginia were able to relate within the lower economic groups both family size and level of household sanitation to the frequency of both past and observed infections

The increased risk related to having older siblings together with a number of isolated observations suggests that an older but preschool sibling often brings the virus into the home Once in the home as indicated in Table 58 virus typically spreads to the other homologously nonimmune household members (92) and provides a challenge exposure which frequently results in the reinfection of those already immune (at least 21 overall) However as Table 58 suggests fecal virus was much less readily detected in the single specimens collected from persons undergoing reinfection (7 of 65 or about 10 %) than in those from persons with primary infection (57 of 136 or about 42) Suggestive evidence also was obtained for spread between adjacent households and within a neighborhood Interestingly this observation could not be confirmed by Melnick and his group[17] in their study of the upper-economic group in Charleston West Virginia Only once were we able to link an episode with some certainty to an instance of paralytic disease in the neighborhood

Including associates as well as index children there were 244 instances of primary infection at an approximately known time Histories taken shortly after the infection episodes were detected revealed no illness in relation to 73 per cent of the infections and only minor febrile illness in 24 per cent In 9 instances (4) aseptic men

ingitis was suggested *in retrospect*. *In no instance* did paralytic disease result. To emphasize further the relative safety of natural immunization under the conditions prevailing in southern Louisiana, we may mention our computation, based on infection rates in our study group as applied to the general population of the area, that about 850 infections were required to produce 1 case of paralytic disease.

INFECTIONS IN THE STUDY GROUP DURING 1956

Observations in 1956, after primary vaccination, were modified to permit more certain detection of alimentary tract infections in that stools were collected twice monthly from all children *under 15 years of age* rather than once monthly from the index child.

All vaccinations were with a single unselected lot of commercially produced vaccine. The sero-immunity status of the group 1 month after the second dose of vaccine is shown in Table 59. Of the 118 children with no antibody before vaccination, 36, 26 and 60 per cent failed to develop antibodies capable of inhibiting the cytopathic effects of Types 1, 2 and 3 viruses, respectively. However, much better de novo development of antibody was observed in those already possessing naturally acquired antibody to 1 or 2 heterologous virus types.

As noted in Table 56, there were 47 episodes of household infection in 1956 among the 118 *households under observation*. Two of these were limited to single individuals undergoing reinfection and would not have been detected by the methods of observation previously employed. The 45 remaining episodes all involved one or more persons experiencing primary infection and may be compared with 52 episodes observed in 1955 prior to vaccination among about 140 households. Evidently primary vaccination did not act to reduce the frequency of household episodes.

Information regarding virus spread within 43 households is shown in Table 60 and should be *compared with the data contained in Table 58* but with two important reservations. First, the 1953-1955 data in Table 58 relate only to households in which the episodes involved the index child; comparable data for the 10 abortive episodes in which the index child escaped being incomplete. If such data could have been included the resulting figures for spread of virus within all households infected prior to vaccination probably would have been very close to those shown in Table 60 for the postvaccination experience (87 % primary infections and 16 % reinfections disregarding age). Second, due to the boosting effect of vaccination on pre-existing *antibody levels*, it is even more probable in 1956 than in 1954-1955 that only a portion of the actual reinfections were detected serologically because of the difficulty in showing a significant antibody rise in persons whose titers already are high. In any event, the reinfections detected in 1956 are noteworthy because in 10 instances they occurred despite presumed reinforcement of the natural immunity by vaccination. Over all, it does not

TABLE 59. NEUTRALIZING ANTIBODY DEVELOPING *DE NOVO** 1 MONTH AFTER 2 DOSES OF SALK VACCINE IN RELATION TO SERO-IMMUNE STATUS *PRIOR TO VACCINATION*

PRE-VACCINATION IMMUNITY	No	POSTVACCINATION IMMUNITY: NUMBER OF PERSONS AND PER CENT WITH TITERS OF 1 x AGAINST VIRUS: 0†	2–5	10–20	40+
		TYPE 1			
None	118	36 (35)	30	28	6
Only Heterologous	97	10 (6)	22	41	27
		TYPE 2			
None	118	26	21	41	12
Only Heterologous	97	8 (3)	14	39	38
		TYPE 3			
None	118	60 (59)	23	13	5
Only Heterologous	94	18 (13)	32	35	15

* These data refer only to development of antibody of the type or types not present prior to vaccination.

† The percentages classed as negative include specimens negative in 1:2 dilution, the separate figures for which are shown in parentheses, and those *heel- or finger*-puncture specimens which were negative in 1:10 dilution, which was the lowest that could be tested.

TABLE 60 VIRAL EXCRETION AND SEROLOGIC RESPONSE TO INFECTION AMONG MEMBERS OF 43* HOUSEHOLDS UNDERGOING EPISODES OF POLIO VIRUS INFECTION IN 1956 AFTER A PRIMARY COURSE OF SALK VACCINE

Homologous Prevaccination Immunity	Age Group (Years)	Serologic Response to Infection Episode	Number of Persons: Total	Number of Persons: Excreting Virus †	Proved Infections: Ratio‡	Proved Infections: %
None	15 and over	None	4	0	[illegible]/6	33
		Yes	2	1		
	Under 15	None	12	3	87/96	91
		Yes	84	81		
	All ages	None	16	3	89/102	87
		Yes	86	82		
Yes	15 and over	None	80	0	8/88	9
		Yes	8	2		
	Under 15	None	40	6	13/45	29
		Yes	7	5		
	All ages	None	120	6	21/133	16
		Yes	15	7		

* Four household episodes excluded because data not complete (2 households) or single individual infected (2 households) was experiencing reinfection rather than primary infection.
† Data as to virus excretion based on specimens collected twice monthly as a routine in case of persons under 15 years of age whereas in case of older persons they depend on single specimen taken soon after infection of young person was detected.
‡ Ratio of number of persons infected to number exposed.

TABLE 61 DURATION OF FECAL EXCRETION OF VIRUS IN CHILDREN AS RELATED TO HOMOLOGOUS IMMUNITY STATUS PRIOR TO INFECTION

Previous Natural Immunity	Vaccination Immunity: Vaccine	Vaccination Immunity: Sero-response	Number of Children Excreting Virus*	Days Duration of Virus Excretion: Observed: Range	Observed: Mean	Estimated True Mean
None	None	None	110†	1–114	24	51
	Primary Only	None	34	1–105	27	47
		Yes	50	1–85	24	42
	All with primary		84	1–105	26	44
Present	Yes‡	Yes & No	15§	1–43	7	12

* As detected by cytopathic rather than pH test.
† Infections occurring in 1954–1955 prior to vaccination.
‡ All but 2 had been vaccinated.
§ Includes 4 members of the households not included in Table 60.

seem that vaccination influenced intrahousehold spread of infection

A final aspect of interest is related to excretion of virus in the feces Of the 87 children undergoing primary infection after vaccination only 3 were not found to be excreting virus As Table 60 further indicates when continuous observation was maintained viral excretion also was commonly found among children who were experiencing reinfection This was the case in 5 of the 7 manifesting antibody rise and in 10 additional children (including 4 not mentioned in Table 60) The data in Table 61 indicate that duration of excretion was significantly reduced in children undergoing reinfection whereas in children experiencing primary infection it did not differ materially as between unvaccinated and vaccinated children or within the vaccinated group as between those who did or did not manifest homologous serologic response to vaccination Finally in Table 62 we have a crude estimate of the levels of fecal infectivity in the first virus positive specimens Not only was there no significant difference related to vaccination in the specimens from primary infections but also rather surprisingly nearly equal fecal infectivity was found in reinfections In summary the primary course of vaccination did not appear to influence either the frequency the duration or the amount of fecal virus excretion in primary infections In contrast prior natural immunity did appear to shorten the period of excretion in reinfections

PRELIMINARY REPORT OF OBSERVATIONS IN 1957 AFTER THE BOOSTER INOCULATION OF VACCINE

The third or booster dose of vaccine was given during January 1957 and bloods were collected 1 month later In Table 63 are presented the data as to neutralizing antibody attributable only to vaccination in the postbooster sera of those children not exposed to intrahousehold infection in the interval since primary vaccination Special interest pertains to those who were completely nonimmune prior to vaccination and failed to respond detectably to the primary course Unfortunately a significant proportion of specimens were obtained by heel or finger puncture and could not be tested in dilution lower than 1 10 The Type 2 response was again the best titers of 1 10 or higher being elicited in all cases and of 1 160 or better in 89 per cent The response to Type 1 was intermediate and that to Type 3 much the poorest the percentages negative in 1 2 or 1 10 dilution being 23 and 50 respectively Apparently few of those who failed to develop Type 1 or Type 3 antibody after primary vaccination were conditioned to respond to the booster inoculum with antibody detectable in 1 10 dilution in spite of the fact that the booster was from a commercially prepared lot of vaccine selected with the advice of the Division of Biologic Standards as being of maximal potency However a very different picture is seen among those who possessed 1 or 2 types of naturally acquired antibody prior to vaccination With but 2 exceptions (both in respect to Type 3 antigen) all responded and very commonly with titers of 1 160 or higher

Data as to infections in the study group during 1957 are complete only for the first 4½ months Over all enteric viral infections were detected frequently with 42 Coxsackie 29 ECHO and 15 unclassifiable viruses but only 6 polio virus infections These latter represented 1 household episode each of infections with Types 1 and 3 viruses The small number of episodes at this stage in the year has little mean

TABLE 62 INFECTIVITY OF THE INITIAL VIRUS POSITIVE STOOL SPECIMENS IN RELATION TO PREINFECTION IMMUNITY STATUS OF THE DONORS

Prior Natural	Vaccination	Number of Persons	Log Infectivity Titer*		
			Mean	Median	Range
None	None	15	4 9	5 0	<2 0–6 5
	Yes	43	4 8	5 0	<2 0–6 5
Yes	Yes + No	6	4 2	5 0	<2 0–5 5

* Titer expressed as log TCD_{50} per Gm of stool and as determined very roughly based on use of 2 culture tubes per tenfold serial dilution It should be noted that, since largest inoculum tested per tube was 0 1 ml of 10 per cent extract of stool the lowest log titer detectable is 2 0 logs per Gm of original stool

TABLE 63 OBSERVATIONS 1 MONTH AFTER THE THIRD OR BOOSTER INOCULUM OF SALK VACCINE AS TO NEUTRALIZING ANTIBODY TO POLIO VIRUSES ATTRIBUTABLE SOLELY TO THE VACCINE*

IMMUNITY PRIOR TO PRIMARY VACCINATION	NUMBER OF PERSONS	PER CENT OF PERSONS WITH ANTIBODY TO VIRUS TYPE INDICATED IN TITERS OF 1 x																	
		TYPE 1						TYPE 2						TYPE 3					
		0†	2–5	10–20	40–80	160–320	640+	0†	2–5	10–20	40–80	160–320	640+	0†	2–5	10–20	40–80	160–320	640+
None	62	23 (8)	5	10	18	27	16	0	0	3	8	55	34	50 (16)	6	13	18	8	5
Only Type 1	19																		
Only Type 2	14	0	0	7	7	43	43	0	0	10	10	53	26	0	10	16	37	21	16
Only Type 3	26	0	0	8	4	23	65	0	0	4	8	38	50	7	0	29	7	43	14
2 heterologous types	10 16 ∠0	0	0	10	0	60	30	0	0	0	31	63	6	5	5	10	25	35	20

These d ta f r nly to the d elopm nt of ant body f the type or type n t p sent prior to the p ima y co se of va ine n person who wer not exposed to infection w t in the househ ld during the 11 month interv l n 1956 between compl tion of the primary c u se nd adm n trat on f th booste noculum

† As in T ble 59

7 Fox J P Discussion of paper by Paul J R *et al*

8 Fox J P Gelfand H M Bhatt P N LeBlanc D R and Conwell D P Immunizing infections with poliomyelitis viruses and sero-immune patterns in southern Louisiana Ann New York Acad Sc 61 968 1955

9 Fox J P Gelfand H M LeBlanc D R and Conwell D P A continuing study of the acquisition of natural immunity to poliomyelitis in representative Louisiana households Am J Pub Health 46 283 1956

10 ——— Studies on the development of natural immunity to poliomyelitis in Louisiana I Over all plan methods and observations as to patterns of sero-immunity in the study group Am J Hyg 65 344 1957

11 Gelfand H M Fox J P LeBlanc D R Observations on natural poliovirus infections in immunized children Am J Pub Health 47 421 1957

12 Gelfand H M LeBlanc D R Fox J P and Conwell D P Studies on the development of natural immunity to poliomyelitis in Louisiana II Description and analysis of episodes of infection observed in study group households Am J Hyg 65 367 1957

13 Hammon W McD Coriell L L Ludwig E H McAllister R M Greene A E Sather G E and Wehrle P F Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis 5 Re analysis of results based on laboratory confirmed cases J Am M A 156 21 1954

14 Howe H A Day by day response to poliomyelitis infection of vaccinated chimpanzees Presented before the Epidemiology Section American Public Health Association 84th Annual Meeting Atlantic City N J Nov 14 1956

15 ——— Studies of active immunogenesis in poliomyelitis I Persistence and recall by homotypic or heterotypic superinfection of neutralizing antibody originally induced in chimpanzees by vaccination or infection Am J Hyg 60 371 1954

16 Howe H A Bodian D and Morgan I M Subclinical poliomyelitis in the chimpanzee and its relation to alimentary tract reinfection Am J Hyg 51 85 1950

17 Isacson P Melnick J L, and Walton M Environmental studies of endemic enteric virus infections II Poliovirus infections in household units Am J Hyg 65 79 1957

18 Koprowski H Norton T W Hummeler K Stokes J Jr Hunt A D Jr Flack A and Jervis G A Immunization of infants with living attenuated poliomyelitis virus J A M A 162 1281 1956

19 Koprowski H Norton T W Jervis G A Nelson T L Chadwick D L Nelsen D J and Meyer K F Clinical investigations on attenuated strains of poliomyelitis used as a method of immunization of children with living virus, J A M A 160 954 1956

20 Lipson M J Robbins, F C and Woods W A The influence of vaccination upon intestinal infection of family contacts of poliomyelitis patients J Clin Invest 35 722 1956

21 Melnick J L Walton M Isacson P and Cardwell W Environmental studies of endemic enteric virus infections I Community sero-immune patterns and poliovirus infection rates Am J Hyg 65 1 1957

22 Paul J R Horstmann D M Deutsch J V and Melnick J L Immunization with killed poliovirus followed by induced infection with attenuated poliovirus *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 141 151 New York New York Acad Sc 1957

23 Rappaport C Trypsinization of monkey kidney tissue an automatic method for the preparation of cell suspensions Bull World Health Organ 14 147 1956

24 Robbins F C Enders J F and Weller T H Cytopathogenic effect of poliomyelitis viruses in vitro on human embryonic tissues Proc Soc Exper Biol & Med 75 370 1950

25 Robbins F C Enders J F Weller T H and Florentino G L Studies on the cultivation of poliomyelitis viruses in tissue culture V The direct isolation and serologic identification of virus strains in tissue culture from patients with nonparalytic and paralytic poliomyelitis Am J Hyg 54 286 1951

26 Sabin A B Present status of attenuated live virus poliomyelitis vaccine J A M A 162 1589 1956

27 ——— Properties of attenuated polio-viruses and their behavior in human beings in Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 113 127 New York New York Acad Sc 1957
28 Salk J A Poliomyelitis vaccination in the fall of 1956 Am J Pub Health 47 1 1957
29 Weller T H Enders J F Robbins F C and Stoddard M B Studies on the cultivation of poliomyelitis viruses in tissue culture I Propagation of poliomyelitis viruses in suspended cell cultures of various human tissues J Immunol 69 645 1952
30 Weller T H Robbins F C and Enders J F Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues Proc Soc Exper Biol & Med 72 153 1949

DISCUSSION

DR. BROWN I do not intend to enter into any discussion of the relative merits of live or killed virus vaccines. Our primary objective is the control of poliomyelitis by whatever method or combination of methods proves to be most satisfactory

Dr Fox has made a beautiful study and one that represents another demonstration of the wholehearted and unselfish co-operation on the part of the test subjects themselves in furnishing specimens over such a long period of time

The work of Dr Sabin is indeed a perfect example of precise thorough and exacting research and demonstrates the extent of attenuation obtained with single plaque strains of virus

Dr Koprowski in his interesting paper shows clearly that persons previously vaccinated with inactivated virus respond to the feeding of attenuated virus with marked antibody production The increased titers obtained were in fact as high as those of some of the persons previously exposed to attenuated virus and fed live virus again after 2½ years

Both Koprowski and Sabin have emphasized the point that vaccination with living virus does seem to prevent the development of a carrier state on re-exposure while previous vaccination with killed virus vaccines does not In the latter instance therefore virus is being recirculated in the community But we must not forget that the vaccinated subjects are protected against paralysis and that more and more individuals are having their immunity re-enforced as a response to the ensuing subclinical infections

Two of the most pertinent questions to be answered are (1) What is the duration of immunity following the administration of killed virus vaccines? and (2) What level of circulating antibodies is necessary for protection against paralysis?

Dr Koprowski has shown that antibodies may persist for 3 to 6 years following administration of attenuated viruses. This is valuable information Although Dr Salk has previously described studies of the duration of antibodies following inactivated vaccines I present the results of the study which we have made in children receiving commercial inactivated vaccines under actual field conditions.

Figure 76 presents the geometric mean antibody titers of a group of school children before and after primary vaccination during the nationwide field trial of 1954 before and after a booster inoculation 1 year later in 1955 and finally their antibody status in 1957 Thirteen of the children were negative to all 3 types before primary immunization in 1954 Their 1955 and 1957 titers are shown in Figure 77 Considering the fact that a 2 year period had elapsed since the booster inoculation the height of the present titers argues that immunity has endured

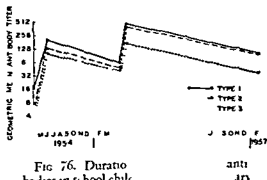
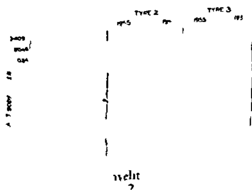
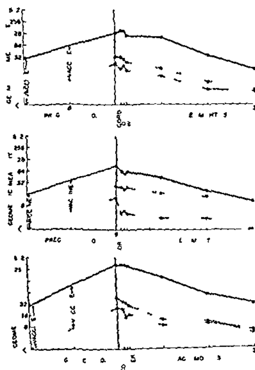


FIG 76. Duratio ... anti
bodies in school chil... ary
and secondary inocu
vaccine (63 subjects—



velit
2



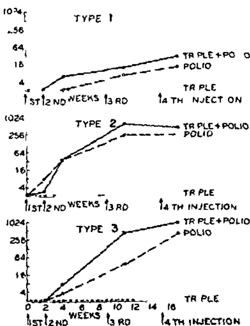


Fig 81 Geometric mean poliomyelitis antibody titers in monkeys 2 weeks after each of 4 vaccine injections

Fig 78 (*Top*) Type 1 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

Fig 79 (*Center*) Type 2 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

Fig 80 (*Bottom*) Type 3 serologic response of pregnant women to poliomyelitis vaccine and passive transfer of antibodies to infants

I would call attention to the data presented by Dr Koprowski on persistence of antibodies in which some of his titers decreased perhaps two to fourfold In a recent publication by Dr Lennette the titers following actual paralytic infection in individuals decreased over 2 years approximately fourfold and the figures agree very closely with ours 2 years following the booster inoculation

Figures 78 to 80 present another type of study which we have conducted on the duration of antibodies passively transferred to their infants by vaccinated and unvaccinated mothers. Figure 78 shows the behavior of Type 1 Figure 79 of Type 2 and Figure 80 of Type 3 antibodies It is of considerable interest that the rate of decline of all whether naturally or artificially evoked in the matter was approximately the same

We are currently engaged in additional research which by its nature requires a killed virus vaccine that is immunization with multiple antigen preparations We have obtained satisfactory responses in laboratory animals inoculated with a preparation containing diphtheria and tetanus toxoids pertussis vaccine and trivalent poliomyelitis vaccine (Fig 81) Certain problems of concentration preparation and preservation remain to be solved as well as of dosage and administration But the results so far have shown considerable promise Perhaps in the near future manufacturers may combine these components and protect children against several diseases with one multivalent vaccine

Prof Dr Dick There are 3 stages in testing live polio virus vaccines The first consists of laboratory tests of the strains to be used

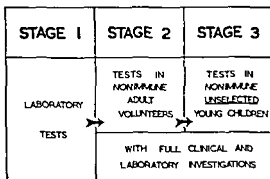


Fig 82 The 3 stages in the testing of live polio virus vaccines

and the second consists of tests of these strains in nonimmune adult volunteers Dr Sabin's rigid laboratory tests of his attenuated viruses justified his proceeding to stage 2 the results of which he has presented to us These first 2 stages are however a preparation for the third stage which consists of feeding the viruses to *unselected* nonimmune young children (Fig 82) This is the most important stage for we know from our studies of Dr Koprowski's Type 2 TN strain that there may be great differences in the multiplication of polio virus in the alimentary tract of children as compared with adults My reason for saying unselected young children is as follows If a group of institutional children devoid of antibodies is taken for testing *oral vaccines these children may have already* been naturally selected for a degree of resistance Dr Koprowski's results with TN Type 2 vaccine which we could not confirm may have been due to the fact that he unwittingly selected children who were naturally poor hosts for polio viruses In the third stage a qualitative and quantitative comparison between the virus *excreted* by vaccinated children and natural virus strains from paralytic and inapparent infections in children is required

For various reasons the tests at stages 2 and 3 should be done at different times of the year for we cannot conclude that the same results will be obtained when live virus is fed in January as in September or at other times of the year in tropical countries

It may be found that virus which is excreted after vaccination spreads from vaccinated individuals to their contacts If spread does *not* occur then all that is required for safety is to show that the virus which the children excrete after vaccination is less virulent than naturally occurring paralytic strains

If on the other hand spread *does* occur and we found this with Dr Koprowski's Type 1 SM strain then it must be shown that either the excreted virus differs from naturally occurring strains and is more attenuated or if the excreted virus is as avirulent as naturally occurring strains then we must be assured that the natural virulent strains do not become virulent on *passing through a community for if they do* then the excreted vaccine viruses may do so too

Finally what antibody level will be produced by avirulent virus vaccines which pass the tests of stage 3? Dr Sabin has not given us any antibody data from his studies in adults It may be that the more highly attenuated strains will behave like the highly attenuated yellow fever or influenza viruses and fail to produce antibodies Are we then between the devil and the deep blue sea—strains like SM Type 1 and TN Type 2 *both* of which after multiplication in the human gut become unacceptably virulent but are antigenic as against strains which may be acceptably avirulent but of poor antigenicity? The titers of antibody which we achieved with the partially attenuated SM and TN viruses were no better than those obtained after 2 injections of formolised vaccine

Dr Sabin has suggested that the immunity resulting *from ingestion of attenuated* strains may be as long lasting as that following natural infections I know of no evidence that durable immunity follows natural infection with non invasive strains Dr Koprowski's claim for the durability of antibody for 6 years refers to vaccination with a strain which produced virulent fecal virus after vaccination *If poor levels of* antibody are produced and the immunity is not durable is this going to be improved with more highly attenuated strains? It may be that the best we can hope for is a *super Salk* vaccine using inactivated purified virus which might be expected to produce higher and more durable levels of antibody than the available formolised vaccine provides

Dr Horstmann The 3 papers have given a comprehensive picture of the problems which we now face in connection with the possible use of live attenuated polio viruses for vaccina

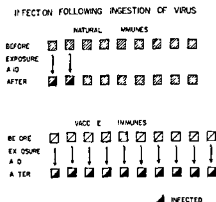


Fig 83 Responses of natural and vaccine immune" persons to ingestion of 10^7.4 TCD of LSc strain of attenuated Type 1 polio virus

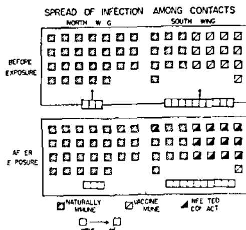


Fig 84 Spread of Type 1 LSc strain of attenuated polio virus among contacts of persons infected following ingestion of virus

tion The work also brings out how much has been learned about human poliomyelitis as a result of investigations concerned with the enhancement of immunity This is particularly apparent in connection with the nature of immune mechanisms in this infection

Because they are easy to define and measure we have concentrated on circulating antibodies as indicators of resistance but more and more evidence seems to be accumulating that antibodies are not the whole story and that probably local immunity or so-called tissue immunity also may play a role The data presented this morning by the 3 speakers all bear on this point Unless the intestinal tract has participated in active infection with live multiplying virus it remains completely susceptible to viral implantation even in the presence of significant antibody induced by killed vaccine

In a human trial of the LSc strain of attenuated Type 1 virus generously made available to us by Dr Sabin we also have observed marked differences between responses of children with vaccine induced antibodies as compared with children with naturally acquired antibodies As shown in Figure 83 when fed a large dose of this strain of virus (10 000 000 tissue-culture infective doses) all 10 children with vaccine-acquired antibodies (100 per cent of those fed) readily became infected excreted virus for a long time developed neutralizing and complement fixing antibodies In contrast only 2 of 9 naturally immune children became infected These 2 excreted virus briefly developed rises in neutralizing antibodies but no CF response

We also measured the degree of spread which might occur when attenuated viruses are fed to children in a closed community Our observations with the LSc strain and more recently with Dr Sabin s KP 34 strain of Type 3 indicate that when these attenuated strains are fed to children in a group all of whom possess antibodies either naturally acquired or vaccine induced the agents spread rapidly and readily to susceptible contacts The speed of spread is indicated by the fact that contacts were already found to be excreting virus 4 to 6 days after the test subjects had been fed

Figure 84 shows a comparison of the rates of spread among vaccine immune and naturally immune contacts in this unit of 69 individuals Essentially 13 infected persons who had been fed virus were introduced into the group What happened was that 1 of 36 or 3 per cent of the naturally immune contacts became infected while for the vaccine immunes the infection rate was 12 of 13 or 92 per cent a figure remarkably similar to the infection rates reported by Dr Fox and by others for infection of fully susceptible children exposed to virus in their homes

TYPE I ANTIBODY LEVEL PRE-EXPOSURE	VACCINE IMMUNE NUMBER	%	NATURALLY IMMUNE NUMBER	%
0 <4	5/5	100	(1/1)	–
4–16	9/9	100	–	–
32–64	8/8	100	2/15	13
128–256	0/1	–	1/20	5
512–1024	–	–	0/9	0

FIG 85 Relation between antibody titer at time of exposure and infection in 69 individuals Asterisk indicates susceptible individual

The role of the level of antibody in conditioning responses of the 2 groups vaccine and naturally immune is considered in Figure 85 In this Type 1 trial only in the range of 32 to 64 neutralizing antibody level were there sufficient persons in each group for an adequate comparison Here 8 of the 8 vaccine immunes became infected while 2 of the 15 or 13 per cent of the naturally immune persons became infected It has been suggested that higher levels of vaccine acquired and naturally acquired antibodies may be capable of preventing alimentary infection At present however there is no indication that this is the case

Taken together the data reported point to the relative nature of immune barriers in poliomyelitis and the dependence of this immunity on a balance or ratio between antibody level dosage of virus virulence of the infecting strain and probably previous experience or lack of it of the alimentary tract with the virus That this naturally acquired immunity is more solid in terms of infection than that acquired from killed vaccine is indicated by the field experience and by the controlled human trials However I agree with Dr Sabin that the results thus far achieved with the killed vaccine in terms of the reduction of paralytic poliomyelitis demand its continued use in countries where economically feasible to see what it will do over the course of years In the meantime however trials on an experimental basis calling for the cautious use of attenuated strains orally also seem warranted

Because of the ease of spread of attenuated viruses the problem of the stability of these strains becomes as important for the community as for the individual fed Evidence has been presented today and our experience agrees with this that even the most highly attenuated strains *in certain individuals may show some tendency* toward increased neurotropism although by comparison with wild strains they remain still highly attenuated

One argument is that it would seem desirable to replace the wild virulent strains which are so *widespread in many areas at all times with* highly attenuated strains This argument has logic and force but the practical problem of how to go about doing this and how to evaluate the results adequately is for the proponents of this plan to provide In highly endemic areas where the wild strains are most prevalent the incidence of paralytic poliomyelitis is very low and the disease is confined to the infantile age group In this situation it would be necessary to feed attenuated viruses to enormous numbers of children over a long period of time to achieve statistically significant results It would also be difficult to administer such a program successfully in underdeveloped areas requiring as it does careful follow up and reporting of cases and extensive testing of excreted virus to find out if the attenuated strains are actually replacing the virulent ones and if so for how long

Nevertheless in spite of the difficulties I for one hope to see such experiments carried out in the near future Perhaps one way to minimize the difficulties would be to use the attenuated strains in areas where epidemics are making their appearance for the first time If attenuated virus vaccine could be administered at the beginning of epidemics particularly those in relatively small population groups perhaps on islands an evaluation of the procedure could be achieved although only after repeated trials of this sort

Another possibility would be a trial in a small country which has had and may expect to have epidemic poliomyelitis and has not yet used killed vaccine on any scale There is much to be said for such a trial at this time using the most attenuated most stable and most antigenic strains This is a bold program but no more so than was the field trial with the killed vaccine and now as then it would seem that the right mixture of boldness and caution are needed

BIBLIOGRAPHY

Brown G C Rabson A S and Schieble J H The effect of gamma globulin on subclinical infection in familial associates of poliomyelitis cases II Serological studies and virus isolations from pharyngeal secretions J Immunol 75 71 1955

Davis D C and Melnick J L Unpublished observations

Dulbecco R *In* Cellular Biology Nucleic Acids and Viruses (Special Publication) vol 5 pp 138 139 New York New York Acad Sc 1957

Horstmann D M McCollum R W and Mascola A D The incidence of infection among contacts of poliomyelitis cases J Clin Invest 34 1573 1955

Isacson P Melnick J L and Walton M Environmental studies of endemic enteric virus infections II Poliovirus infections in household units Am J Hyg 65 29 1957

Lipson M J Robbins F C and Woods W A The influence of vaccination upon intestinal infection of family contacts of poliomyelitis patients J Clin Invest 35 722 1956

Prof Lépine In countries where poliomyelitis is rare and where it remains strictly infantile the resistance normally acquired by the population comes at the same time from a spontaneous absence of early infection by the 3 types of the viruses and the prevalence of relatively attenuated strains which infect these populations in conditions which ensure a fairly equal distribution of the different antigenic types Hence the temptation for the research worker to try to reproduce artificially what nature does spontaneously in countries where this natural immunization predominates this of course provided that the method chosen does not involve greater risks than those which result from spontaneous infection

This obvious limitation led us to think in terms of using attenuated viruses no longer as a method of primary vaccination but as a means of enabling us to re-enforce possibly at a second stage and for prolonged duration the protection obtained earlier at a first stage through a vaccination by an inactivated virus

Our tests have taken place on the chimpanzee exclusively They were reported recently at the Conference on Cellular Biology Nucleic Acids and Viruses which was held at the Academy of Sciences of New York January 7 to 9 1957 If these tests have indeed confirmed the theoretical possibility of obtaining such a re-enforcement of the basic immunity by the oral administration of the attenuated polio virus for the two following reasons our conclusions have stressed the express reservations concerning the practical possibilities of applying this

1 The vaccinated subjects become virus carriers which does restrict the possible application of this method to communities or to groups where all the individuals have been given a prior basic immunity

2 The strains of the polio virus which have been totally attenuated only yield an irregular and short-duration response with the vaccinated animals

The findings of Dane Dick and their co-workers which have been published since dealing with vaccinated subjects in Northern Ireland with a modified attenuated virus have confirmed the first of these reservations by showing the true danger of relapse to the primitive virulence of the vaccine strain by the adaptation of the virus vaccine to the intestinal medium and its transformation into a wild strain

The second reservation which is even more serious deserves some comment Our monkeys indeed have been vaccinated and have received their basic immunity with a vaccine prepared on the basis of the method advocated by the Pasteur Institute This is inactivated vaccine made of a mixture of the inactivated culture of the 3 types of virus with the following main characteristics (1) the use of selected strains with a high antigenic power nonpathogenic by subcutaneous or intramuscular route (2) culture medium entirely synthetic and absorption of the culture on synthetic resin ensuring the absence of serum proteins or animal proteins from the finished vaccine (3) inactivation of the virus in 2 stages through an action of 2 antiseptics at low doses bringing about total inactivation in 72 hours and finishing the operation at a low temperature which guarantees the integral preservation of the antigenic power (4) no filtration of the cultures on ultrarefined candles which facilitates retention of all the virus without any loss of antigens (5) mixture of the monovalent lots in the final polyvalent vaccine not by volume but in terms of their antigenic potency for each of the lots

Under such conditions such a vaccine used for a basic immunization with a dose of 3 subcutaneous injections 3 weeks apart determines with the animal as with man an immune response so strong that the peroral administration of attenuated strains of the polio virus is not followed by reaction and in fact there is no increase of the antibody titer

If enough time is allowed to elapse say a year for the basic immunization due to the inactivated virus to begin to weaken and then we begin to adminis er through the mouth an attenuated virus we are confronted with the following alternatives which we have not been able to avoid so far

Either the attenuated virus then administered *has lost sufficient neuropathogenicity so that it* may be considered as innocuous in which case it evoked a weak and irregular response the antibodies increasing in an inconstant manner and the titer remaining in any case lower than that which you would get through a simple booster injection of the inactivated vaccine or the virus then is still capable of provoking a response in the organism and evidently has adequate antigeni power However in that case we always deal with strains which have not completely lost their neuropathogenic potency and therefore we cannot consider them as giving us the sufficient security guarantee for them to be applied without discrimination to man

As it was impossible to avoid this alternative we gave up following this approach in our tests Such *test might well be taken up again with* strains the certain innocuity of which would allow for their introduction in the organism by a parenteral route

One should not forget that the digestive tract is after all an invaginated portion of the exterior medium and that it is not strictly speaking an integral part of the organism It is host normally to a number of bacteria and viruses against which the organism reacts only if they show a certain aggressiveness

If the attenuated viruses which we administer orally are sufficiently attenuated not to be aggressive any longer there is no valid reason to expect the organism *to become immunized* against them If they preserve sufficient aggressiveness to provoke an antigenic response they keep in that way the very characteristics of a potentially dangerous virus

That is why in spite of the positive value that we do recognize generally speaking in vaccination by means of attenuated viruses and in spite of the results already obtained in experiments in the present circumstances we must give some preference to vaccination by means of inactivated vaccines provided that vaccin s giving necessary guarantees of security and antigenicity such as those we have be used They *alone will give us the results that* we think we can achieve

Immunization Against Poliomyelitis in the Light of Existing Immunity of Populations

DR A M M PAYNE

I have tried to present the information that I have been able to collect regarding the status of immunity of populations measured by different methods in different parts of the world, knowledge which is necessary for the guidance of health authorities in deciding whether or not to apply vaccination and, if so, to which age groups it should be applied.

A decision to introduce immunization against a communicable disease depends on the danger of exposure to the disease and the presence of a susceptible population. The danger of exposure to poliomyelitis obviously depends in the first place on the presence of the 3 types of poliovirus. The results of serologic surveys and virus isolations have shown that all 3 types of virus are present more or less continuously in all populations examined except in relatively small and isolated communities where the infection is unable to maintain itself through lack of susceptibles. Here the infection dies out and is reintroduced at intervals which may be quite lengthy, in which event the well known virgin soil epidemics occur. The apparent absence of poliomyelitis in a community may be due to two totally different situations. On the one hand the infection may be so highly endemic that infection occurs at a very early age and causes very few or no clinical symptoms. As is well known, such a situation occurs in the tropics. On the other hand the absence of cases may be due to the absence of virus, as has been demonstrated in the Arctic. In the former situation immunization is not necessary, whereas the latter population is in a most precarious position because the introduction of a virulent strain would be followed by a devastating epidemic.

These are the two extremes, but closely analogous situations exist in different sections of large continental population groups and even perhaps in the same town. In fact this is of considerable importance to those who are planning poliomyelitis control at the national level.

Assuming the presence of the 3 types of poliovirus in a country, susceptibility is reflected in the incidence and the age incidence of disease. I would emphasize the importance of studying paralytic cases rather than all cases. The clinical diagnosis of nonparalytic poliomyelitis is not a specific diagnosis, since a variable and often considerable proportion, up to about two thirds, is not due to poliovirus, and inclusion of these cases might have a large influence on the results.

When estimating the susceptibility of populations by the study of the incidence of disease, greatest stress should be laid on the age incidence, since the actual incidence depends on other factors in addition to susceptibility and, as is well known, changes greatly from year to year. Figure 8[illegible] shows typical examples of the age incidence found in different parts of the world. The figures used are the age specific attack rates expressed as percentages of the maximum age specific attack rate, and they are plotted on a logarithmic scale.

In Sweden the attack rate over 25 years of age is still nearly 50 per cent of the maximum rate which occurs in the age groups 1 to 4 and 5 to 9, whereas in Israel the attack rate at the age of 5 is one tenth of the maximum attack rate which occurs at the age of 1. In Sweden the rate in the first year of life is only 15 per cent of the maximum, whereas in Israel it is 80 per cent of the maximum rate. However, there are special epidemiologic circumstances in Israel which may account for this very high incidence in the first year. The other examples show the intermediate positions.

Figure 8[illegible] also shows the differences in age distribution of cases found within a country in different areas. I have given only one example. In the United States the relative rates in the 5 to 9 and older age groups in the south are markedly lower than in the west. Other examples are found in other countries. In Switzer-

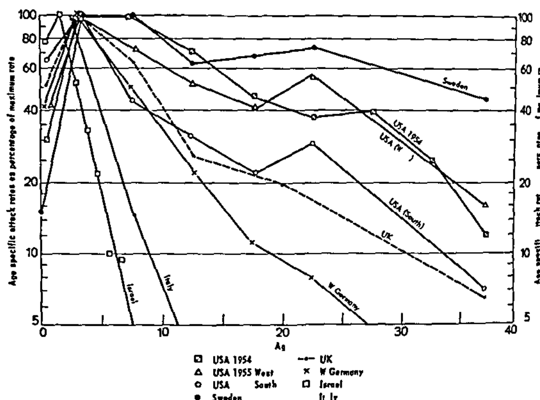


Fig 86 Age incidence in some countries

land a similar difference is to be found between different cantons for example in Lucerne and Berne there is a higher incidence in older age groups than in Geneva and Fribourg In some countries a similar difference has been shown between urban and rural areas the latter generally having a higher incidence in the older age groups

I have no satisfactory figures to show the different age distribution of cases in different population groups in the same town but the observations of Walton and Melnick suggest that it may be considerable the highest socioeconomic groups showing a pattern similar to that of Sweden and the lowest approaching that of Egypt The results of serologic surveys support this view The importance of this in designing vaccination programs is obvious The differences in different parts of a country may sometimes be great enough to justify the complication of immunizing different age groups and in some countries consideration certainly should be given to immunizing older age groups in upper-socioeconomic groups

Obviously in addition to age incidence the mean incidence over a period of years must be a major factor in influencing a decision as to whether or not to introduce immunization In countries with good reporting systems this presents no difficulties although it may be well to stress again that only paralytic cases should be considered In other countries reported figures may be quite meaningless A good example of this was Egypt Between 1944 and 1951 the mean annual number of cases reported was 95 In the course of their classic studies in Cairo, Paul and his colleagues estimated that the real attack rate in that city lay in the neighborhood of 4 to 8 per 100 000 population The correctness of this estimate has been shown by subsequent events

You heard yesterday the official delegate from Egypt estimate the attack rate as about 9 per 100 000 population According to the official figures received by WHO the rate is about 5.2 per 100 000 population practically all of which are paralytic But I think it is safe to assume that official reporting is still incomplete e pe

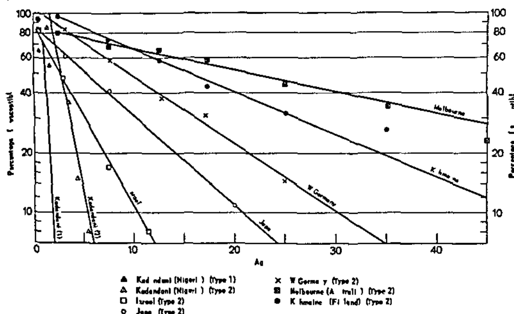


Fig 87 Percentage of susceptibles to Type 2 virus in some areas (Linear scale of age)

cially in rural areas The median incidence of polio in the United States in recent years has been of the order of 20 per 100 000 of which between one third and one half were nonparalytic giving an incidence of about 10 to 15 paralytic cases per 100 000 population There fore it may be said that the problem of poliomyelitis in Egypt is not really much less than it is in the United States a very different assessment to the assessment generally made This is doubtless because in Egypt other health problems overshadow poliomyelitis as a cause of disease

Statistics regarding the incidence of poliomyelitis must be interpreted differently from those for many other communicable diseases where the outcome is recovery and sequelae are rare or unimportant In poliomyelitis anything more than minimal paralysis is permanent so that the morbidity is in effect cumulative

A recent study by Payzin in Turkey suggests that what has been found in Egypt may be true of other countries in that part of the world The suggestion here is of course that there is a large number of undetected cases in many countries and now that we have an effective means of exercising a large measure of control over paralytic disease the problem of poliomyelitis must be reassessed

How is this to be done? Although a large measure of success was obtained in Egypt by efforts to improve reporting there are many areas in the world where this is quite impracticable at present It would seem however that valuable information might be gained by properly designed clinical surveys such as those carried out by Paul in Cairo looking for a residual paralysis The second method of estimating susceptibility and assessing the poliomyelitis situation is by means of serologic surveys for neutralizing antibodies which provide a record of the past experience of the population

Thanks to the generous co-operation of members of the WHO Expert Advisory Panel on Virus Diseases and of many other virologists many of whom are present today I have been able to collect about 100 surveys in more than 40 different countries The presentation and the interpretation of such a mass of data from such diverse sources is a matter of considerable difficulty Different methods of selecting the study population different laboratory technics and different serum dilutions have been used Some surveys are quite inadequate in size whereas a

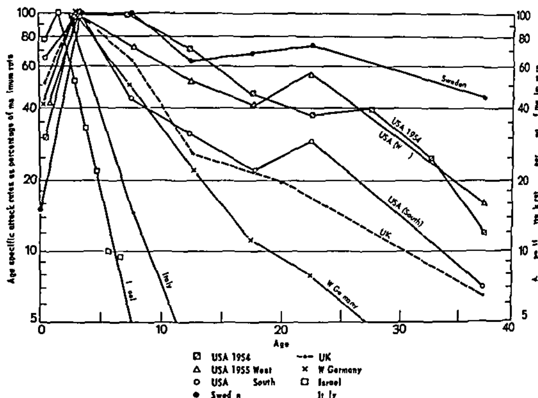


Fig 86 Age incidence in some countries

land a similar difference is to be found between different cantons for example in Lucerne and Berne there is a higher incidence in older age groups than in Geneva and Fribourg In some countries a similar difference has been shown between urban and rural areas the latter generally having a higher incidence in the older age groups

I have no satisfactory figures to show the different age distribution of cases in different population groups in the same town but the observations of Walton and Melnick suggest that it may be considerable the highest socio-economic groups showing a pattern similar to that of Sweden and the lowest approaching that of Egypt The results of serologic surveys support this view The importance of this in designing vaccination programs is obvious The differences in different parts of a country may sometimes be great enough to justify the complication of immunizing different age groups and in some countries consideration certainly should be given to immunizing older age groups in upper socioeconomic groups

Obviously in addition to age incidence the mean incidence over a period of years must be a major factor in influencing a decision as to whether or not to introduce immunization In countries with good reporting systems this presents no difficulties although it may be well to stress again that only paralytic cases should be considered In other countries reported figures may be quite meaningless A good example of this was Egypt Between 1944 and 1951 the mean annual number of cases reported was 95 In the course of their classic studies in Cairo Paul and his colleagues estimated that the real attack rate in that city lay in the neighborhood of 4 to 8 per 100 000 population The correctness of this estimate has been shown by subsequent events

You heard yesterday the official delegate from Egypt estimate the attack rate as about 9 per 100 000 population According to the official figures received by WHO the rate is about 5 ? per 100 000 population practically all of which are paralytic But I think it is safe to assume that official reporting is still incomplete espe

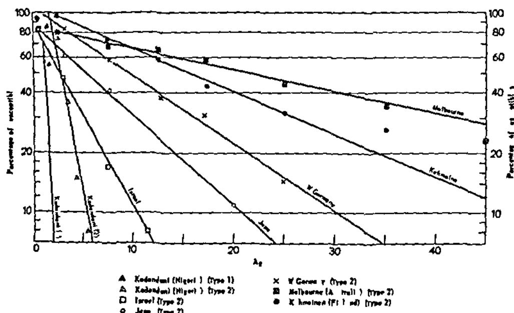


Fig 87 Percentage of susceptibles to Type ? virus in some areas (Linear scale of age)

cially in rural areas The median incidence of polio in the United States in recent years has been of the order of ?0 per 100 000 of which between one third and one half were nonparalytic giving an incidence of about 10 to 15 paralytic cases per 100 000 population Therefore it may be said that the problem of poliomyelitis *in Egypt is not really much less than it* is in the United States a very different assessment to the assessment generally made This is doubtless because in Egypt other health problems overshadow poliomyelitis as a cause of disease

Statistics regarding the incidence of poliomyelitis must be interpreted differently from those for many other communicable diseases where the outcome is recovery and sequelae are rare or unimportant In poliomyelitis anything more than minimal paralysis i permanent so that the morbidity is in effect cumulative

A recent study by Payzin in Turkey suggests that what has been found in Egypt may be true of other countries in that part of the world The *suggestion* here is of course that there is a large number of undetected cases in many countries and n w that we have an effective means of exercising a large measure of control over paralytic disease the problem of poliomyelitis must be reassessed

How is this to be done? Although a large measure of success was obtained in Egypt by efforts to improve reporting there are many areas in the world where this is quite impracticable at present It would seem however that *valuable information might be gained by properly* designed clinical surveys such as those carried out by Paul in Cairo looking for a residual paralysis The second method of estimating susceptibility and assessing the poliomyelitis situation is by means of serologic surveys for neutralizing antibodies which provide a record of the past experience of the population

Thanks to the generous co-operation of members of the WHO Expert Advisory Panel on Virus Diseases and of many other virologists many of whom are present today I have been able to collect about 100 surveys in more than 40 different countries The presentation and the interpretation of such a mass of data from such diverse sources is a matter of considerable difficulty Different methods of selecting the study population different laboratory technics and different serum dilutions have been used Some surveys are quite inadequate in size whereas a

few are very large indeed In addition there is the well known variation in the results of a laboratory test both between laboratories and within laboratories on repetition of the test To illustrate I will mention the work of Olin and Wesslen who found that on retesting 200 sera 14 1 per cent gave a different result To this must be added sampling and other errors mentioned above so that it is evident that most surveys can be regarded as showing only a rough approximation to the real situation In view of this I have adopted a method of approximation which seems to have some advantages

In Figures 87 and 88 the vertical scale shows the percentage of susceptibles remaining at a given age plotted on a logarithmic scale The horizontal scale is the age generally on a linear scale though there is sometimes an advantage in using a logarithmic cale for age as well Below 10 per cent the errors in these surveys and the small figures make divergence from the straight lines rather unimportant—rather without much meaning When many surveys are plotted in this way they fall rather closely along straight lines As you will see the fit is quite good

Figure 87 shows susceptibles to Type 2 virus in Melbourne in Kuhmoinen in Finland West ern Germany Japan Israel and Kadandani which is a village in Nigeria These I have selected as being representative of the various patterns of the serologic survey scene Fifty per cent of these populations are susceptible at the age of 22 in Melbourne 15 in Kuhmoinen 9 in Germany 5 in Japan and 2 in Israel and Kadandani The 10 per cent level is not reached at all in Melbourne and Kuhmoinen It is reached at the age of 30 in Germany 21 in Japan 10 in Israel and 5 in Kadandani Below the 10 per cent level as I have already said the small numbers make the changes in percentages of doubtful significance But it does seem that in *most surveys the complete absence of susceptibles* is unusual and there is often a tendency for the percentage to show an increase in adult life Whether this is due to loss of antibody below the level of sensitivity of the test or to some other cause is a matter for speculation These 6 surveys show the most usual patterns in areas of different endemicity All intermediate patterns may be found The most rapid loss of susceptibility that I have been able to find was to Type 1 virus in Kadandani One hundred per cent of 2 year-olds were already immune This presumably was due to a recent epidemic wave *of infection but apparently* there were no cases of paralysis However even

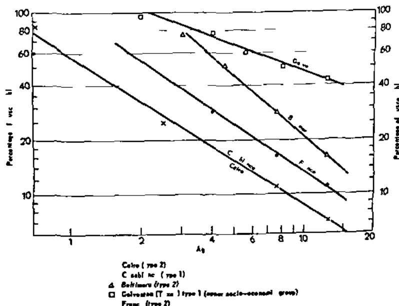


Fig 88 Percentage of susceptibles (Logarithmic scale of age)

TABLE 64 SURVEY OF GROUP I

Prototype Israel 50% <5	10% <10
Belfast	Chile Type 2
Londonderry	Cuba Havana Type 2
Glasgow (lower)	Eritrea
Glasgow (upper) Type 1	Sudan
Italy (Palermo)	Egypt Cairo Type ?
Italy (Naples)	French Morocco Casablanca
Italy (Milan Brescia, Novara)	Nigeria Kadandani
U S Galveston (lower)	Madagascar
U S Texas (lower) Type 2	Mauritius
U S Phoenix (lower)	Liberia
U S Los Angeles (lower) Type 2	Belgian Congo
Canada St Augustin Type 2	India Bombay
Canada Roberval Type ?	Turkey
Uruguay Canelones	Aden Type 2
Venezuela Caracas	Tahiti
Brazil Rio de Janeiro (lower)	Raiatea

TABLE 65 SURVEY OF GROUPS 2 AND 3

Group 2 Prototype Japan 50% 3 6	10% 10 20
N Ireland (counties)	U S Baltimore Type 2
England (1954)	U S Louisiana
Liverpool	U S Charleston (lower)
Betws y Coed	U S Cincinnati Type ?
France (provinces)	U S Thomasville
France (Paris Assistance publique)	Canada Montreal
Belgium (Brussels)	Uruguay Montevideo
Belgium (Flanders)	Korea Type ?
Switzerland (Geneva)	Okinawa Type
Serbia Types ? and 3	N Australia (aborigines)
Group 3 Prototype W Germany 50% 6–10	**10% 20–30**
Freiburg	Iceland Type 2
Marburg	Rumania Bucharest, Type 2
Munich	Hungary Szeged Type 2
Italy (Genoa)	U S Galveston (upper)
Sweden (Norrbottens lan)	U S Miami Type ?
Finland (H Lanki)	Brazil Rio de Janeiro (upper)
Finland (Joensuu)	Hong Kong
Finland (Kemijarvi)	Singapore

in this highly endemic area a few individuals over the age of 40 showed no antibody to 1 or 2 types although in no instance was there no antibody to all 3 types over the age of 1 year

On the basis of this figure it is possible arbitrarily to divide the majority of surveys into 5 groups.

Group 1 those in which the percentage of susceptibles diminish as rapidly or more rapidly than in Israel

Group 2 those lying between the graphs for Israel and Japan

Group 3 those between Japan and Western Germany

TABLE 66 SURVEY OF GROUPS 4 AND 5

GROUP 4 PROTOTYPE KUHMOINEN 50% 10–15 10% 10–50	
Glasgow (upper) Types 2 and 3	Finland Aland Is
Hamburg	U S Allegheny
Switzerland (Vaud)	U S Winston Salem
Sweden Stockholm Type 1	U S Phoenix (upper)
Sweden Got o Bohus Lan Type 1	S Australia
Sweden Goteborg	W Australia Como
Sweden Kronoberg	New Zealand Otago
GROUP 5 PROTOTYPE MELBOURNE 50% >15 10% >50	
Sweden Stockholm Types 2 and 3	Canada Eskimos
Sweden Got o Bohus Lan Types 2 and 3	Alaska Eskimos
U S Charleston (upper)	Serbia Type 1

Group 4 those between Western Germany and Kuhmoinen

Group 5 those acquiring antibody more slowly than in Kuhmoinen

In placing surveys into various groups greatest emphasis has been placed on the age at which 50 per cent susceptibles remain In some surveys only that figure was available since a wide-enough age group was not examined In others the 10 per cent level was approached early but not reached

In most surveys of adequate size the graphs for all 3 antibody levels fall into the same group In a few instances where the differences are very marked the types are specified Where only Type 2 antibodies have been tested this is also specified In deciding whether to place a survey in one of two neighboring groups if it is a borderline case preference is given to the findings for the Type 2 antibody However it should be remembered that this method of presentation is a rough approximation only It is quite impossible to present all these results in full detail and the inherent errors of these surveys must constantly be borne in mind

In Table 64 the surveys of the first group are shown I would like to point out that the upper socioeconomic division in Glasgow falls into this very early acquisition of antibody with respect to Type 2 Types 2 and 3 for Glasgow we will find in Group ? which we come to later The patterns found in the lower socioeconomic groups of highly developed countries are quite comparable with those found in the most backward countries Table 65 shows Groups 2 and 3 Here you will find that for Serbia Types 2 and 3 fall into Group 2 but later in Group 5 you will find Serbia Type 1 which is very very strange Hong Kong and Singapore both rather surprisingly fall into Group 3 although in both instances it was the lower socioeconomic groups which were examined In Table 66 are Group 4 and Group 5 Much as you would expect countries with a severe polio problem fall into this Group 5

Where the necessary information was available I have also studied the percentage of susceptibles remaining at the age below which 90 per cent of all cases have occurred or at which the attack rate has fallen to one tenth of the maximum rate There is some variation but in the majority of instances between 20 and 30 per cent of susceptibles remain to each virus type at that age In areas where the antibody pattern is that of Group 1 fewer susceptibles remain than in areas where antibodies are acquired more slowly But evidently few cases are to be expected in the population with less than 20 per cent susceptibles

Agreement between susceptibility estimated on the basis of age incidence and by serology is reasonably close Very close agreement is not to be expected because in most instances the two populations are not identical one perhaps being a small sample of a particular town the

Fig. 89. Susceptibility as estimated by age incidence of disease compared with estimate by antibody surveys.

other being the incidence in a whole province or a whole country. Figure 89 gives 3 representative examples. There is an error in this figure: the antibody Types 1 and 2 for Western Germany have been interchanged. You will note that the acquisition of antibody has roughly the same slope as the decrease in incidence of cases, the cases decreasing a little more rapidly in each instance than the rate of acquisition of antibody. I am afraid that time does not permit any more expansion of that subject.

The interpretation of the significance of the individual antibodies in protecting in this disease is dependent on whether there is cross protection between the different types of virus. It has long been held that previous infection with Type 2 virus gave some cross immunity to the other types. That was discussed by Lepine at the last Conference, so there is no need to review earlier evidence further, but since then, as Sabin has shown, the monkeys challenged with Type 1 virus less frequently develop paralysis if they already had Type 2 antibody produced by vaccination or by infection. Salk yesterday read to us some of his work on the interrelationship of the Types 1 and 2 and 2 and 3 having antigenicity in common.

Material which was kindly sent to me by Brown supports this view. In the 1954 trial an extensive survey showed that in an area 50 per cent of the study population had antibodies to Type 2 before vaccination. A study of antibodies in cases occurring during the trial confirmed by virus isolation showed that out of [illegible] paralytic cases from which Type 1 virus was isolated only 2 had antibody to Type 2. If there was no relationship between the presence of Type 2 antibody and paralysis due to Type 1 one would have expected that 50 per cent of the cases would have had Type 2 antibody. Thus there are 24 cases with Type 2 antibody missing from the paralytic group.

Figure 90 shows the results of serologic surveys in different socioeconomic groups in Charleston, W. Va. The straight lines approximate the rates of development of Type 2 antibody in the 2 socioeconomic groups, upper and lower. The lower group I would point out up

POLIOMYELITIS
INDIA
TOWN

C
and
G
slowl
In
est en
which
surveys
wide-eno
others th
early but
In most s
for all 3 anti
In a few ins
very marked
only Type 2 ant
also specified I
survey in one of
is a borderline cas
findings for the Ty
should be remembere
sentation is a rough
quite impossible to pr
full detail and the in
surveys must constantly l

In Table 64 the surveys
shown I would like to poin
socioeconomic division in Gl
very early acquisition of anti
to Type 2 Types 2 and 3 for
find in Group 2 which we con
patterns found in the lower
groups of highly developed count

) 1956.

Type 2 antibodies but they are not enough Consequently there are considerable contingents of populations in our country who need protection against poliomyelitis and we are going to give them this particular protection by means of vaccination with the vaccine produced in our country

Note Dr Chumakov g e h Iscus i n in Ru an He uppl ed the inter reters with an En h h text fr m wh ch this draft was taken. Unfortunately any add ti ns that may have been made could not be tr n lated

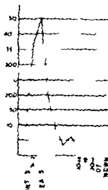
Dr Gharpure I present a few facts which will explain existing conditions on the basis of which I have been carrying out my work *Figure 91* is a map of India and I have depicted by triangles the areas where paralytic poliomyelitis exists The triangles with the darkened upper corner show areas where it is notifiable Thus it will be seen that out of a large country the disease is notifiable in only one third of it The notifiable paralytic cases generally have been about 1 000 each year I roughly calculate that the incidence would be about 10 per 100 000 In the child population it may be about 4 or 5 times as high

There are ups and downs during these 11 years which are more or less constant for the last 8 years *Figure 92* shows the age incidence These figures are from Bombay but they more or less apply to the rest of the country as well Here you will find that it is restricted to the child population the peak being at 1 to 2 years

Figure 93 shows the seasonal incidence It is concentrated in a few months May to September When the figures over a number of years are plotted along with the rainfall one sees some rather interesting results (Fig 94)

There are ups and downs during these 11 years and the peaks to some extent correspond to high rainfalls We have never been able to ex

	949	9 0	195	52	1953	19	1955	1956	OTAL
0-6 MONT S		5	8			3	8	10	5
6 2	83	3	8	5	23	0	29	6	358
YR 2 RS	6	40	—	33	38	10	3	9	4 9
2 RS 3Y S	0	27	5	2		3	16	25	
3 4 RS	8	9	0	8	4		9	6	8
5 RS		3		2				8	33
8 9 S	0	0	5	5	8			8	67
0-14	3		0	0		3		6	
5 19 RS			0	0	2			3	
20 & ABOVE	0	0		3		8			2
AGE NOT D	0		0		0				
TO AL	253	33	5	9	2	259		2 3	3 0



	1949	50	195	952	19 3	1954	55	1956	TOT
J N	0	5	3	5	9	4	8	3	37
FEB	0	9		5	6			5	33
M R	0		4	18	9		5	4	53
A	2	4	2	2	8	2	8	8	68
M				26	5	8	15		9
UN		10		20	6	23	16	39	40
JUL	76	23	9		15		23	6	273
UG	94			5		73	5	29	28
SE	29	18	5	2	7	28		23	23
OC	3	2	15			3		20	98
NOV			5			10	2	0	52
DEC	3	0	9	5	3	3	5	9	57
TO L	253	33	74	69	82	459		2 3	310

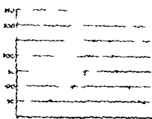


Fig 92 (*Top*) Age incidence of poliomyelitis in India
Fig 93 (*Bottom*) Seasonal incidence of poliomyelitis in India

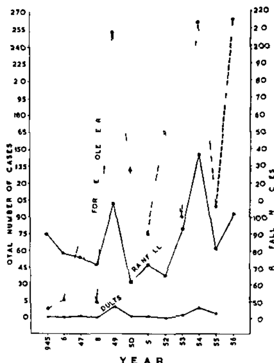


Fig 94 Number of cases of poliomyelitis compared with incidence of rainfall (Bombay 1945 1956)

plain exactly what this means On an all India basis I have been able to collect some sera through the Armed Forces Medical College which is a training center where young men from 18 to 20 attend Two hundred fifty nine sera have been tested so far and they show antibodies against all 3 virus types

More particular studies were carried out in Bombay Figure 95 shows quite a complex set of data I will not refer to all of it You will find that paralytic cases are scattered all over Each year in the same regions cases recur and over 200 cases have been occurring from 1949 onward In a total of some 1 400 cases so far only 47 have been in persons above 14 Last year a small group of 3 000 children were investigated One hundred twenty four sera were studied (Fig 96) which showed that about 25 persons up to the age of 12 are susceptible One hundred fifty two polio viruses have been isolated of these 117 were Type 1 13 were Type 2 and 22 were Type 3 It was rather remarkable that in the first 6 years Type 1 was the most common Type 2 came up occasionally but, in 1955 1956 Type 3 paralytic poliomyelitis has occurred more frequently and on the borderlines of areas which previously were affected

I would refer to another study carried out in the town of Dohad (Fig 97) 300 miles from Bombay where in 1957 an epidemic of poliomyelitis occurred with 112 cases in children in a population of 25 000 Subsequently there have been 10 to 12 cases each year but poliomyelitis persists Last year we started a house to house survey the data are not yet complete but the sera study showed the same results namely that up to the age of 10 or 12 25 per cent of the children are susceptible (Fig 98) Here again I might stress that poliomyelitis continues to exist

The Andaman and the Nicobar Islands experienced a poliomyelitis epidemic in 1949 a report of which came up before the Second International Congress Then for years nothing was heard Suddenly in December 1956 I received a telegram from the Government of India saying that poliomyelitis had broken out on an adjacent island Dr Seal went up to the spot and I procured the materials for virus isolation and serology—more than 100 sera which show a good acquaintance or experience with all the 3 types of the virus I will not analyze the data completely but I cite this as an instance that after a gap of several years poliomyelitis had broken out again

I have mentioned these facts to explain the various types of existing conditions In Bombay the same number of cases continues in Dohad after a large sized epidemic a small number of cases continues in Andaman a Bombay type epidemic continues and I begin to feel that unless measures of active immunization are undertaken poliomyelitis will not be controlled Today in India vaccine is not manufactured and it is not being used on a large scale but on a voluntary basis for whoever wants it The public and the doctors are vaccine minded and I am glad about it

Prof Giovanardi Before expressing some observations on the application of vaccination against poliomyelitis in Italy I shall refer briefly

to the behavior of neutralizing antibodies as an index of the condition of natural immunity of a population

This study was performed on groups of population belonging to middle or low hygienic sanitary and socioeconomic classes of north and south-central Italy—Biella Brescia Milan Novara Naples Bari Cosenza and Taranto The results are summarized in Figures 99 100 and 101 As can be seen the neutralizing antibodies of the 3 types of polio virus occur with great frequency at birth decline progressively to a minimum at the end of the first year of life and then increase till reaching a maximum at the age of 5 to 6 years At such age in all groups of population the number of subjects with anti

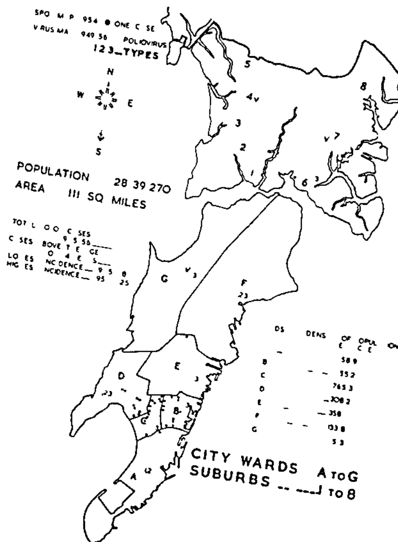


Fig 95 Incidence of poliomyelitis in greater Bombay

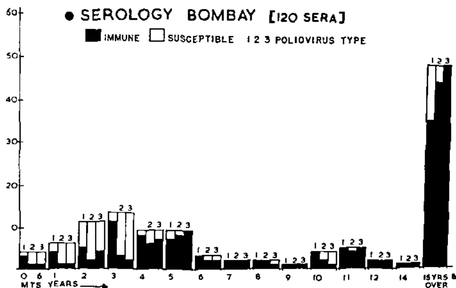
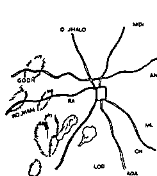


FIG 96 (*Top*) Results of serology tests in Bombay



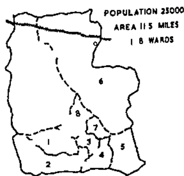
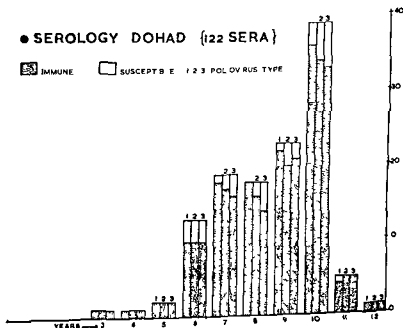


FIG 97 (*Left*) Map of Dohad and its adjoining villages
(*Right*) Map of Dohad showing its wards

FIG 98 (*Bottom*) Results of serology tests in Dohad



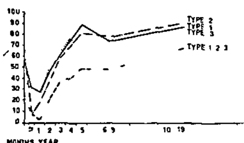


Fig 99 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Milan Brescia and Novara in relation to age

bodies for the 3 types of polio virus is from 50 to 80 per cent and the number of subjects with antibodies for Type 1 Type 2 or Type 3 may reach 90 per cent and more

According to official data about two thirds of paralytic poliomyelitis cases in Milan and Brescia and in all of Italy occur in the first 3 years of age

The behavior of the complement fixation antibodies and for comparison of the neutralizing antibodies in 200 children of Naples and Cosenza is shown in Figures 102 and 103

Under 2 years of age only a few children—5 to 10 per cent—have complement fixation antibodies The number of children with these

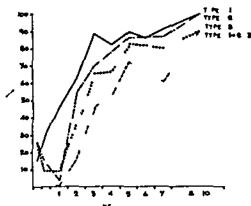


Fig 101 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Naples Taranto Bari and Cosenza in relation to age

antibodies then increases rapidly reaching rather high values about 50 per cent at 5 years

In conclusion I would call attention to the following points

1 In Italy the age range of those least protected by natural immunity is rather short and probably extends from the first year of life to the fourth and fifth year

2 It follows that the application of vaccination is to be considered mainly for the children of the early ages up to the fifth year

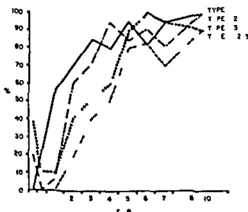


Fig 100 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in Naples in relation to age

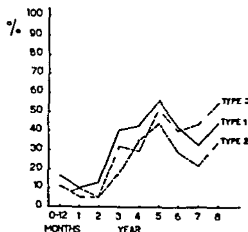


Fig 103 Behavior of complement fixation antibodies against the 3 polio virus types in a group of subjects in south Italy in relation to age

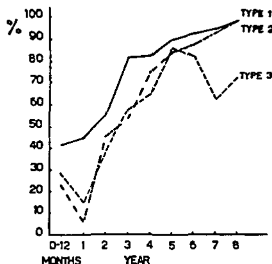


Fig 103 Behavior of the neutralizing antibodies against the 3 polio virus types in a group of subjects in south Italy in relation to age

3 In classes of population more refined from hygienic and economic standpoints or in isolated population groups in south *Italy* it is possible that the age period not protected by the natural immunity is wider and therefore that in these conditions vaccination deserves a more extensive application

4 The behavior of complement fixation tends to show that in Italy infection and reinfection from polio virus occur with a certain frequency at least till the age of 8 This is probably a *favorable condition in which to employ vaccination* with killed virus because the immunity roused by primary vaccination can be strengthened by these infections This is not only for the amount of antibodies but also for the quality because it must be established whether the antibodies from killed virus from all protection standpoints are of the same potency as antibodies from living virus—from virus which multiplies in the human body

BIBLIOGRAPHY

Albano A Boll Ist sieroterap milanese 35 339 and 526 1956

Brancato P Picciotto L and Posteraro G Riv Ist sieroterap ital 31 236 1956

Giovanardi A Monaci V and Bonetti F Boll Ist sieroterap milanese 34 772 1955

Melnick J L Proc Soc Exper Biol & Med 89 131 1955

Melnick J L Paul J R and Walton M Am J Pub Health 45 429 1955

Oddo F G and De Grazia G Riv Ist sieroterap ital 31 39 1956

Salk J E Ann New York Acad Sc 61 1023 1955

Dr Goldblum I shall restrict my comments to three major points the antigenic crossing between the polio virus types the immunologic response of infants to vaccination with a killed vaccine and the influence of mass immunization on the future relationship of polio virus to man

The antigenic crossing among the 3 polio virus serotypes and especially between Types 1 and 2 advanced by Sabin and others and recently re-emphasized by Salk may be of greater importance in the epidemiology of poliomyelitis than has been previously anticipated and may have a bearing on mass immunization programs Most of our information on the distribution of immunity to poliomyelitis has been gathered from surveys of Type 2 antibodies which undoubtedly is due to the fact that technics for measuring these antibodies have been available for over a decade Since practical methods for measuring Type 1 and 3 antibodies have become available only recently not enough information is as yet on hand Fortunately as pointed out by Dr Payne the occurrence of Type 2 antibodies is a fairly good index of the susceptibility of populations to paralytic poliomyelitis

Table 67 is from a survey of polio antibodies carried out recently in our country illustrating this point Antibodies to Type 1 polio virus develop very early and by the age of 18 months two thirds of the infant population have had experience with this type of virus The acquisition of Type 2 antibodies comes later and lags behind Type 1 by almost 1 year Thus by the age of 18 months only 19 per cent have Type 2 antibodies and two thirds is reached only 12 months later In Israel Type 1 virus is responsible for over 80 per cent of paralytic poliomyelitis and all this occurs in infants and young children from 6 to 36 months of age

Therefore it seems to me that the prevalence of Type 2 antibodies may be not only an important index for measuring the susceptibility of a population to poliomyelitis but also that its

Table 67 Results of Antibody Tests of Israeli Infants and Children to the 3 Polio Virus Types Prevaccination Bloods

Age Group (Months)	No Tested	Antibodies To Type 1	%	Type 2	%	Type 3	%	Without Antibodies To Any Type	%	Antibodies To All 3 Types	%
3–6	85	15	18	16	19	12	14	53	61	3	5
7–12	90	33	36	9	10	23	25	42	47	3	4
13–18	84	56	66	16	19	29	37	18	21	9	11
19–24	68	53	77	28	41	38	56	6	9	18	26
25–30	65	56	86	44	67	52	80	2	3	34	52
31–36	52	46	88	41	78	47	90	0	—	33	63
37–48	37	35	95	33	89	32	86	1	3	30	81

endemicity may be of primary importance in determining the epidemicity of Type 1 virus and the resulting occurrence of epidemics of paralytic polio

A situation different from that in Israel prevails in other countries like Nigeria, Morocco and other subtropical areas where Type 2 polio virus is highly endemic and at the age of 1 year over 50 per cent have already been infected with this virus

Basic immunization with killed virus vaccine especially in infants and young children results primarily in the development of high titer Type 2 antibodies. Thus on mass vaccination an infant population is quickly transformed from a Type 2 nonimmune to a Type 2 immune population and this by itself without considering the Type 1 response may be of importance in preventing Type 1 epidemics.

This is now the situation in Israel. Serologic studies indicate the development of Type 2 antibodies to a high degree and rather low for Type 1. Nevertheless there is little paralytic polio this year as compared with previous years.

Although the immunologic response of infants to vaccination with a killed virus vaccine may not be satisfactory we have to face it in all countries where polio is of the infantile type. Only future experience will answer the numerous problems involved in the vaccination of this low age group problems such as the exact age at which to immunize, what amounts of vaccine should be administered, what schedule used and so on. Without having these answers on hand we should proceed to immunize infants shortly after birth say at the age of 3 to 4 months since thus we will be able to prevent a substantial number of cases of paralytic poliomyelitis.

In what measure will mass immunization of a small part of the population—that of infants and very young children—influence the circulation of the different polio virus serotypes? We know and have been reassured this morning that intestinal infection may proceed almost undisturbed in a naturally immune or a partially vaccinated population. However will this continue for years to come or will the picture ultimately change and shall we create a polio virus free population? This is by far one of the most important problems which those responsible for public health will have to face in the future.

Dr Monaci: To give the right judgment on the immunity situation of the Italian population and on an eventual application of a vaccination against poliomyelitis researches have been made on the distribution of the 3 polio virus types and on the incidence of neutralizing antibodies in different age groups.

I shall report on the etiologic results while those of the serologic researches will be reported by Dr C io anardi.

The diffusion and the distribution of the 3 polio types has been studied in different epidemiologic conditions on paralytic polio pa

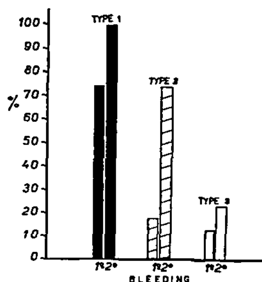


Fig 104 Behavior of the neutralizing antibodies in contacts (epidemic outbreak in Brescia 1953)

tients on population groups (noncontacts) in absence of paralytic polio cases in interepidemic periods on epidemic community outbreaks (contacts)

The results are summarized in Tables 68 to 71 and Figure 104

From our researches on the diffusion of the 3 polio virus types in the Italian population one can state the following

1 Most of the paralytic polio cases are caused by Type 1 virus frequently cases are caused by Type 2 *and rarest are the infections caused by* Type 3

Table 68 Polio Virus Types Isolated from Paralytic Polio Patients in Different Italian Districts from 1953 to 1957

Districts	Virus Type 1	Virus Type 2	Virus Type 3	Total
Lombardy	97	32	7	136
Piedmont	17	2	0	19
Trieste	2	0	0	2
Tuscany	0	3	0	3
Umbria	2	0	0	2
Campania	37	12	7	56
Basilicata	1	0	0	1
Apulia	5	0	0	5
Calabria	0	0	1	1
Total	161	49	15	225

Table 69 Frequency of Polio Virus Types in Cases of Paralytic Polio in Milan Brescia and Novara

Year	Strains of Type Isolated 1	2	3	Total
1953	23	10	1	34
1954	30	0	0	30
1955	23	19	2	44
1956	37	5	4	46
Total	113	34	7	154

Table 70 Polio Virus and Other Cytopathogenic Agents Isolated from the Stools of 900 Healthy Children (Noncontacts)

Polio		Coxsackie		ECHO		Adenovirus		Cytopathogenic Unidentified Agents
Strains No	Type	Strains No	Type	Strains No	Type	Strains No	Type	No
3	2	4	A	10	7	1	1	10
2	3	1	B	1	8	1	2	
						1	5	
						1	6	
5		5		11		4		10
0 5%		0 5%		1 2%		0 4%		1 1%

TABLE 71 ISOLATION OF POLIO VIRUS FRO I CONTACTS EPIDEMIC OUTBREAKS IN INFANTILE COMMUNITIES OF BRESCIA (FROM TYPE 1) AND FLORENCE (FROM TYPE 2)

CITY AND YEAR	NO OF SUBJECTS EXAMINED		SUBJECTS WITH VIRUS IN STOOLS No	%	TYPE OF VIRUS ISOLATED
Brescia 1953	Patients	7	5	70	1
	Contacts	65	33	50	1
	Staff	8	1	12	1
Florence 1955	Patients	2	2	100	2
	Contacts	17	13	76	2
	Staff	3	1	33	2

P ralyt pol

2 Also in nonepidemic periods in absence of paralytic polio cases the virus causes inapparent infections in the population

3 Noteworthy is the isolation from healthy subjects and from infantile gastro-enteritis cases of Type 3 virus which in observed regions in these last years resulted rarely enough in para lytic polio cases The presence of Type 3 virus as a latent infection agent may explain in part the high incidence of neutralizing antibodies in the healthy people an inciden e which as has been noted is not much less than that found for Types 1 and 2

4 In contacts the latent aborti e infections may reach 50 to 80 per cent and more

5 In contacts it is possible to show the pres ence of antibodies neutralizing the type of the virus causing the infection in 100 per cent of the cases

In conclusion the antipolio va cination prob- lem may be summarized as follows

1 The large exposure to the irus and thus the high incidence of pontaneous infections allow natural immunity to be reached by our population in their first years of life

2 As paralyti polio strikes with greater fre quency children from (months to 3 to 4 years vaccination against poliomyelitis should be made at the beginning of the se enth month of life or during the following 5 months

3 Vaccination for all the children of these age groups even it desirable does not seem to be imminent in our country owing to economic and organization difficulties

BIBLIOGRAPHY

Felici A and Archetti I Ann Ist sieroterap ital 31 233 1956

Giovanardi A Monaci V and Bonetti F Boll Ist sieroterap milanese 34 42 1955

Oddo F Riv Ist sieroterap ital 30 17 1955

Terni M Terni Pons V and Lo Monaco G B Riv Ist sieroterap ital 31 231 1956

PROF OKER BLOM As Dr Payne pointed out the need for vaccination may be different even in different population groups in the same coun try In Finland antibody studies have been performed which seem to show this clearly I am therefore going to present a few illustra tions which summarize some of these studies

Altogether about 2 500 blood samples were collected in 1953 and 1954 in the regions men tioned in Figure 105 and were tested by Dr Lapinleimu and myself in our laboratory As was mentioned previously Finland has a polio- myelitis morbidity ranging from 8 per 100 000 to about 70 in epidemic years However some local epidemics with a far greater incidence have occurred (Fig 10() and such Type 1 epidemics happened to occur during or after the sampling in 3 out of the 5 regions shown

The immunity against Type 1 virus in these regions is shown in Figure 107 I have tried to use the same method as Dr Payne in showing the number of susceptibles—probably not with good success—but the 50 per cent susceptibility is reached in Helsinki and the Kemijarvi dis trict at an age between 5 and 9 years, but on

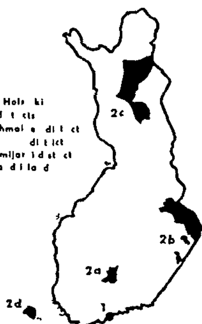


Fig 105 Districts investigated in 1953 1954

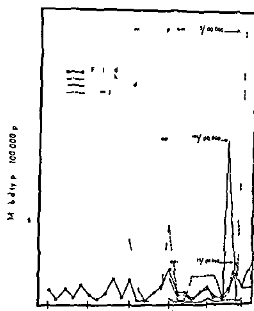


Fig 106 Annual rates per 100 000 population of notified cases of poliomyelitis (Finland Helsinki Aland Islands and Kemijarvi)

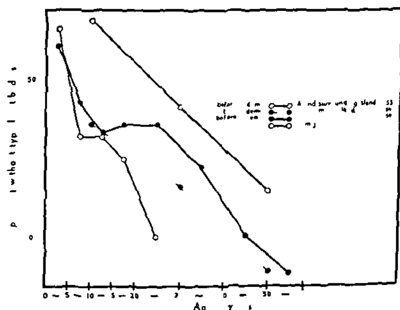


Fig 107 Frequency of persons without Type 1 poliomyelitis antibodies in Aland Islands Helsinki and Kemijarvi (Aland figures from Oker Blom and Strandstrom Ann med exper et biol Fenniae 34 186 Helsinki and Kemijarvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

Aland not until between 20 and 30 years The 10 per cent susceptibility again is reached in Kemijarvi at an age of about 20 whereas it is reached in Helsinki at an age between 40 and 50 and on Aland not at all

The age distribution of cases occurring during the epidemics which followed corresponded fairly closely to the curves in Figure 108 Thus in the Kemijarvi epidemic mostly children under 9 years were affected In Helsinki the age distribution was similar to that in many other countries in Europe with a change toward higher age groups On Aland there was a high inciden e of cases in the upper age groups similar to the epidemics on for instance St Helena described by Dr Gear

Thus there seems to be in our country apparently, very different demands for vaccination in different parts of the country In some parts a vaccination of only children below 10 years would be enough whereas in other parts vaccination of everybody up to an age of 40 or 50 would be necessary

Vaccine is not manufactured in Finland and for several reasons a large scale vaccination program cannot be carried out Therefore it would be important for us to know in which regions and in which age groups the vaccination would be needed most urgently For that reason another study of about 2 500 blood samples has been initiated This study was sponsored by the Presidentti Paasikivi Tohtori Wenner-Gren in lapsihalvaus tutkimus rahasto and samples were collected from different parts of the country by using random sampling methods worked out by Dr Penttinen The tests have been carried out in 3 laboratories and have been completed recently but unfortunately are not yet ready for presentation However it is hoped that this study together with the already performed antibody studies and the epidemiologic data from different parts of the country may help the authorities to make plans for vaccination on a sound basis

BIBLIOGRAPHY

Gear J H S Poliomyelitis (WHO Monograph) p 31 New York Columbia 1955

Lapinleimu K Ann med exper et biol Fenniae 34 Supp 11 1957

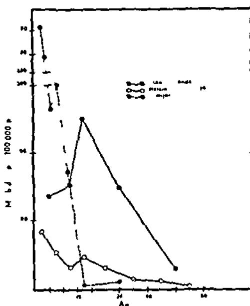


Fig 108 Age incidence of poliomyelitis in Aland Islands (1953) Helsinki (1954) and Kemijarvi (1955) (Aland figures from Oker Blom Ann med exper et biol Fenniae 34 1 0 Helsinki and Kemijarvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

Oker Blom N and Strandstrom H Ann med exper et biol Fenniae 34 186 1956

Dr Salk In view of the important question of the economics of immunization I asked Dr Gear if he would let me present some slides which show a comparison in the antibody response after 2 doses of Reference Vaccine A In the first instance (Fig 109) the symbols on the left indicate the antibody response to 1 cc given subcutaneously the symbols in the middle indicate the response to 1 cc ¼ cc or 1/16 cc given intramuscularly and the symbols on the right the response to 1/10 cc given intradermally If you look across the slide you will see that there is some advantage to the smaller dose when given by the intradermal route

N te Dr Salk was not [illegible] ed for th [illegible] but reque [illegible] be he [illegible]

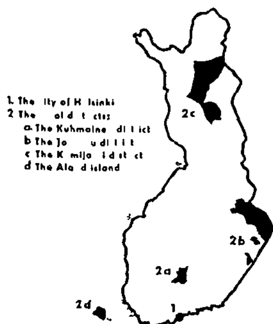


Fig 105 Districts investigated in 1953 1954

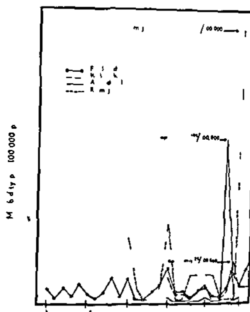


Fig 106 Annual rates per 100 000 population of notified cases of poliomyelitis (Finland Helsinki Aland Islands and Kemijarvi)

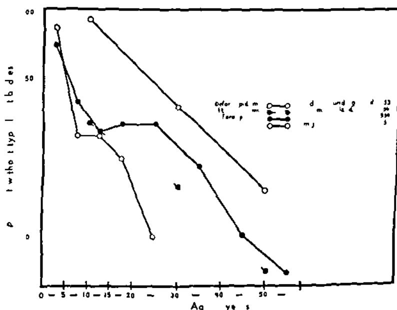


Fig 107 Frequency of persons without Type 1 poliomyelitis antibodies in Aland Islands Helsinki and Kemijarvi (Aland figures from Oker Blom and Strandstrom Ann med exper et biol Fenniae 34 186 Helsinki and Kemijarvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

Aland not until between 20 and 30 years The 10 per cent susceptibility again is reached in Kemijarvi at an age of about 20 whereas it is reached in Helsinki at an age between 40 and 50 and on Aland not at all

The age distribution of cases occurring during the epidemics which followed corresponded fairly closely to the curves in Figure 108 Thus in the Kemijarvi epidemic mostly children under 9 years were affected In Helsinki the age distribution was similar to that in many other countries in Europe with a change toward higher age groups On Aland there was a high incidence of cases in the upper-age groups similar to the epidemics on for instance St Helena described by Dr Gear

Thus there seems to be in our country apparently very different demands for vaccination in different parts of the country In some parts a vaccination of only children below 10 years would be enough whereas in other parts vaccination of everybody up to an age of 40 or 50 would be necessary

Vaccine is not manufactured in Finland and for several reasons a large scale vaccination program cannot be carried out Therefore it would be important for us to know in which regions and in which age groups the vaccination would be needed most urgently For that reason another study of about 2 500 blood samples has been initiated This study was sponsored by the Presidentti Paasikivi Tohtori Wenner-Gren in lapsithalvaus tutkimus rahasto and samples were collected from different parts of the country by using random sampling methods worked out by Dr Penttinen The tests have been carried out in 3 laboratories and have been completed recently but unfortunately are not yet ready for presentation However it is hoped that this study together with the already performed antibody studies and the epidemiologic data from different parts of the country may help the authorities to make plans for vaccination on a sound basis

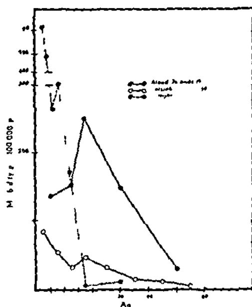


Fig 108 Age incidence of poliomyelitis in Aland Islands (1953) Helsinki (1954) and Kemijarvi (1955) (Aland figures from Oker Blom Ann med exper et biol Fenniae 34 170 Helsinki and Kemijarvi figures from Lapinleimu Ann med exper et biol Fenniae 34 Supp 11)

BIBLIOGRAPHY

Gear J H S Poliomyelitis (WHO Monograph) p 31 New York Columbia 1955

Lapinleimu K Ann med exper et biol Fenniae 34 Supp 11 175

Oker Blom N, and Strandstrom H Ann med exper et biol Fenniae 34 186 1956

DR SALK In view of the important question of the economics of immunization I asked Dr Gear if he would let me present some slides which show a comparison in the antibody response after 2 doses of Reference Vaccine A In the first instance (Fig 109) the symbols on the left indicate the antibody response to 1 cc given subcutaneously the symbols in the middle indicate the response to 1 cc, ¼ cc or 1 16 cc given intramuscularly and the symbols on the right the response to 1/10 cc given intradermally If you look across the slide you will see that there is some advantage to the smaller dose when given by the intradermal route

N T Dr Salk was not f ... at the ... session but requested th t he be ... a few ...

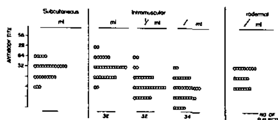


Fig 109 Different routes of inoculation Type 1 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

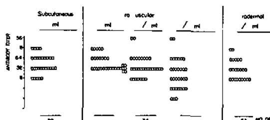


Fig 110 Different routes of inoculation Type 2 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

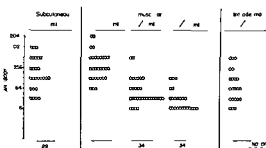


Fig 111 Different routes of inoculation Type 3 antibody response to Reference Vaccine A 2 weeks after 2 doses given 2 weeks apart

The next figure (Fig 110) shows similar data for the Type 2 response you can exercise your own judgment in its interpretation

Figure 111 shows the antibody response to Type 3 Here again we see the slight advantage to 1 cc given intramuscularly but it is clear that a small dose given intradermally seems to have a greater effect than a correspondingly small dose given by the intramuscular route

The next figure (Fig 112) shows the results

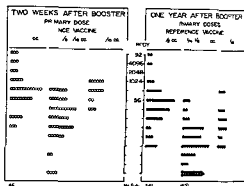


Fig 112 Comparison of intramuscular and intradermal routes for primary vaccination Distribution of Type 1 antibody titers following booster dose 1 year after primary (booster dose = 1 cc Vaccine J i m) (In this figure and Figures 113 and 114 the circles on the left indicate antibody levels 2 weeks after a constant booster dose in all groups where primary injections consisted either of 2 1 ½ ¼ ⅛ or 1/16 cc vaccine given i m or 1/10 cc given i d Data for groups given 2 1 or ½ cc are considered together and groups given ¼ ⅛ or 1/16 are similarly combined for comparison with groups given 1/10 cc i d The solid dots on the right indicate antibody levels 1 year after the booster in the same groups)

observed in these same persons who were given a booster dose intramuscularly with 1 cc of Vaccine J 1 year after the primary—if I may use the word—sensitization with 1/10 cc intradermally or to ¼ ⅛ 1/16 intramuscularly or the larger dose of 2 1 or ½ cc intramuscularly Two weeks after the booster dose the response was somewhat greater for those primarily sensitized with 1/10 cc intradermally as compared with those who had an average of about the same dose *intramuscularly* The same persons were then bled 1 year after the booster dose without any further intervening inoculations You will observe the extent of decline comparing the left and the right column and you will see that all of them had Type 1 antibody 1 year later The sizes of the groups are indicated at the bottom The smaller intramuscular dose was followed by a considerable decline with a number in the zero range in the interval of 1 year

If one has vaccine of low potency such as an *order of magnitude of ¼ ⅛ or 1/16 cc* and one

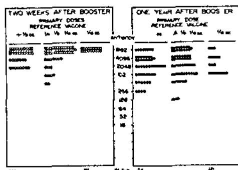


Fig 113 Comparison of intramuscular and intradermal routes for primary vaccination Distribution of Type 2 antibody titers following booster dose 1 year after primary (booster dose = 1 cc Vaccine J i m)

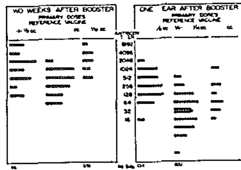


Fig 114 Comparison of intramuscular and intradermal routes for primary vaccination Distribution of Type 3 antibody titers following booster dose 1 year after primary (booster dose = 1 cc Vaccine J i m)

then gives 1/10 cc of such vaccine intradermally one can expect a somewhat lesser effect intradermally than is shown here at the end of the year While there is an advantage to the intracutaneous route when equal volumes are administered the fact that one can give a larger volume intramuscularly provides some slight advantage in compensating for weak vaccine

What this means is another matter I refer strictly to the numerical value for antibody Figure 113 shows the Type 2 response here again the very high order of magnitude This is perhaps the super vaccine to which someone referred today

The last figure (Fig 114) shows the Type 3 response indicating again the advantage and also the comparisons

Dr Paul Certainly Dr Payne has shown us how different the problems are and the rest of the members of this panel have also indicated how the different factors of incidence age groups and levels of society are involved in poliomyelitis This clearly means that the problems of immunization are different in these various places. The panel has agreed that the formalinized vaccine is desirable that it can be made safely and that its use is to be recommended especially in interepidemic or so-called pre-epidemic periods although now it appears also that during epidemics the vaccine should be used

The cost of this vaccine is high If it was not high we would not have needed this session this afternoon because much time has been spent on seeing how it should be rationed There may be some countries even which will wish to wait and try the use of an attenuated vaccine in the hope that at least a field trial will be made so that its *value could be assessed There was a suggestion* made this morning in favor of another Francis field trial and all that goes with it to give some idea as to the value of an attenuated vaccine

Dr Payne's Groups 1 and 2 were to be ideal for this particular sort of trial However some of us rabid epidemiologists look with alarm on the disappearance of nonvaccinated populations We have to get our controls somewhere and we will try to keep our eyes open for them in the last few years before they are gone

Granted that the vaccine is desirable though expensive and in short supply some countries should make their estimates not only on the basis of past experience but also on predictions as to what might happen in the next decade In some countries local cases are still few and epidemics are unknown It might appear that such countries would be ill-advised to go into any major or expensive vaccination program in view of the meager returns However I hope that it is not presumptuous to point out and Dr Chumakov mentioned this also that countries can have a rude awakening such as happened in Costa Rica in Jamaica in the British West Indies where within the past 3 years poliomyelitis epidemics of great severity have appeared precipitously with an abrupt change from the infantile paralysis of

our parents and grandparents to that of modern epidemic polio And once modern poliomyelitis has appeared the subsequent pattern seems to be an irreversible one unless perhaps it is influenced by artificial immunization

As long as I am putting myself in the unenviable position of a prophet what are the diagnostic signs in some of these countries which feel secure at the moment with low rat s of poliomyelitis but might be on the eve of becoming afflicted with modern epidemic poliomyelitis? First I think we should turn to features other than antibody studies namely let us not forget about the changing ways of life in various places and the rather crude indication of the record of infant mortality At the last International Poliomyelitis Conference Dr Payne presented this thesis that low infantile mortality rates were often associated with high poliomyelitis rates and he insisted that there was no cause or relationship which I think is correct although his data testified just as eloquently that there is an extraordinary association In some of these countries in which the infant mortality rate is dropping from 100 to 75 this trend would seem to be a preliminary indication that something might happen

A second feature is the study of the age distribution of the cases It may seem foolish in many places to try to analyze statistically the age distribution of a handful of cases and yet as early as 6 or 8 years before the most recent epidemic in Jamaica the shift began and when th figure of 20 per cent over the age of 10 appeared I think it was time to be watchful

Finally we come to the question of the antibody surveys and the extent to which they may be used as an indication We should not lay too much stress on that particular aspect valuable as the surveys are and perhaps the only measurement that could be made When it comes to interpreting the results of the antibody surveys one may recognize that it is not the antibody level alone that indicates whether the population is immune or ripe for an epidemic It is probably the ratio between antibody level and an unknown figure which represents dosage or availability of virus or amount of virus What would be a normal level of antibody for school children in the cities of the United States as Dr Melnick has shown might be a low level in a tropical area where in terms of dosage of the polio virus to which the population is exposed it might be much higher I think we should recall Dr Gear s remarks made at previous Congresses that poliomyelitis may be essentially a tropical disease perhaps based on the fact that facilities for its spread do exist in tropical countries to a degree that may not be true elsewhere

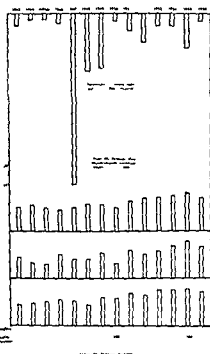
I do not want to belittle the surveys but I am sure that the antibodies are not everything We heard much this morning about the possibility of local immunity something that cannot be measured but certainly should be dealt with The only thing I would suggest in the planning of the use of the Salk vaccine is that it should be remembered that one of the most characteristic features of this particular disease is its tendency to change.

Dr Henneberg Following Dr Payne s suggestion it is more correct to speak of susceptibility rather than of immunity even though this concept is not unambiguously characterized by lack of neutralizing antibodies against the 3 types of poliomyelitis virus In serology positive specific reactions are better indicators than negative ones since the latter depend strongly on reaction threshold values which fluctuate from day to day In the neutralization test a serum which is still positive at a serum dilution of 1 4 should be used concurrently as a control

INDICATIONS FOR INOCULATION

Generally valid statements about an epidemic situation in a large area can scarcely be made this can be done only for a narrowly limited time and place

Ever since 1945 West Berlin has been in a situation unusual for a metropolis The usual fluctuations of population have been absent contacts with the environs have been limited greatly and in brief everything which admittedly serves as a starting point for virus dissemination has been minimized In addition the reconstruction of the city automatically eliminated unhygienic conditions Since the epidemic of 1947 and the postepidemic conditions of 1948 1949 there has been no poliomyelitis epidemic in Berlin which might have served to spread the polio viruses and consequently the prerequisites for epidemic contamination have been lacking The data presented below should be regarded in this light. The morbidity included both paralytic and non paralytic cases which was possible in Berlin because clinically speaking all cases charted were



Poliomyelitis Incidence
per Year per 100 000 Inhabitants
From 1940-1945 for Berlin (per 3 500 00)
From 1946-1956 for West Berlin (per 2,300 000)

Year	Total Incidence	Per 100 000 Inhabitants	
1940	63	1 8	
1941	4 4	13 5	
1942	263	7 5	
1943	143	4 0	*Berlin*
1944	61	1 7	
1945	55	1 6	
1 7 –31 12			
1946	45	3 0	
1947	1255	54 0	
1948	418	18 0	
1949	404	1 1	
1950	51	[illegible] 0	
1951	115	5 0	*West*
1952	[illegible]09	9 0	*Berlin*
1953	85	4 0	
1954	86	4 0	
1955	244	10 6	
1956	54	[illegible] 3	

Fig 115 Morbidity of poliomyelitis in Western Berlin as function of the number of persons without neutralizing antibodies (up to 1945 Berlin after 1946 Western Berlin)

diagnosed as polio by considering the differential diagnosis involved The limitation of the morbidity index to paralytic cases only which Payne considers necessary in a comparable investigation is a procedure dictated solely by necessity The statistical classification of a single definite degree of severity of a disease even of a special case of the disease is unusual especially since we know that various internal and external factors have a decisive influence on the clinical picture

We show the relation of morbidity per 100 000 for the years 1943 1956 to the negative neutralization tests expressed as percentages of the subjects tested The figures are classified by age classes 0 to 14 years and by virus types.

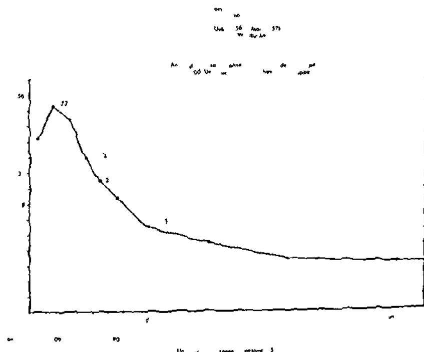
In 194 1949 an epidemic of major proportions swept over all of Berlin The effect of the pandemic—which was reported by Salin to be of Type 1—was reflected in the percentage susceptible Children born before or during the epidemic exhibit a lower percentage of susceptibility than those born later However the differences are so very slight that one cannot really speak of an unusual alteration in percentage negative by a wave of immunization emerging during the epidemic in the age classes concerned The percentage susceptible to Type 3 apparently was not affected increasing at a constant rate throughout the year of the epidemic

Those susceptible to all 3 types were classified by age or age groups. The constant and relatively high percentage negative to all 3 types fell mainly in the higher age classes being about 18 per cent in the age group of 9 to 19 years and 17 5 per cent in the group 30 years and older With regard to the present epidemic situation and the concomitant virus dissemination to be expected we may predict that the number of persons without antibodies will become larger as the younger age cohorts grow older

I think it would be better not to picture the situation as is usually done in the form of a graph To do so would be to prejudice a development which need not necessarily appear through out. For regarding convenience of exposition we may assume that the percentage of susceptible

in the cohort which is now say 14 years old remains the same in this cohort as it ages

In order to get an idea of the effectiveness of immunization throughout definite professional groups the medical personnel were tested in the clinic The relatively high proportion of Type 1 susceptibles is astonishing amounting to 26 anti body negatives in a total of 67 people Also note worthy is the distribution of high antibody titer (1 512 and higher) among workers in the polio and the typhus laboratories and among mothers with children under 10 In 23 of these subjects there were 17 with a neutralization test for 1 of the 3 virus types of 512 or higher while the 31 members of the Institute staff *all* had titers below 512



NUMBER OF PERSONS WITHOUT NEUTRALIZING ANTIBODIES FOR TYPES 1 2 OR 3

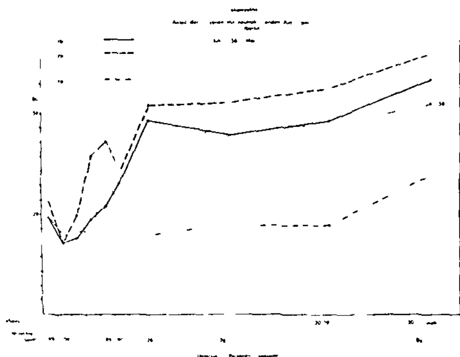
AGE	PERSONS EXAMINED	1	%	2	%	3	%
0–1	99	72	72 7	67	67 6	72	72 7
1 2	109	87	80	87	80	85	78
2 3	109	86	78 9	76	69 7	84	77
3–4	116	85	73 2	65	56	89	76 7
4–5	89	62	70	46	51 2	57	64
5–6	90	57	63 3	54	60	61	67 7
6–7	100	51	51	32	32	57	57
7–8	90	51	56 7	49	54 4	54	60
8–9	74	43	58 1	29	40	33	44 6
9–10	59	29	50	24	40 7	31	52 5
10–11	55	24	43 6	27	50	29	52 7
11–12	59	28	47 4	18	30 5	30	50 8
12–13	71	38	53 5	23	32 4	33	46 5
13–14	55	28	50 9	24	43 6	23	41 8

Total 1175 Persons Examined

FIG 116

In Berlin the number of persons with incomplete immunity seems to be increasing, placing the city in the group which Dr Payne has named the Melbourne Group. The statistical data show no decrease in antibodies with increasing age an observation however which may not be valid beyond the present situation. A loss of immunity would manifest itself in the appearance of second cases of the disease among the older age groups.

In addition when I found only 78 paralytic second cases of poliomyelitis in the literature (Dagulf) to date this small number of cases took on significance. Perhaps they may serve to shake the false security against recurrence of the disease which is now so widespread. The conditions under which paralytic polio due to 1 of the 3



Poliomyelitis

Number of Persons With Neutralizing Antibodies in West Berlin

Age	Persons Examined	1	%	2	%	3	%	1 + 2 + 3	%
0 1 0	99	[illegible]	1	3?	3? 1	2	?7 1	6	[illegible]
1 ? 1	109	[illegible]	0 1		0 1	?4	22.?	4	3 ?
3	109	3	21 1	30	[illegible] 5	[illegible]5	[illegible]9	5	4 6
3 4 3	116	31	[illegible]7	51	44 0	[illegible]	3 3	9	[illegible]8
4 5 4	89		30 3	43	48 3	3	36 0	1[illegible]	13 4
5 6 5	90	33	36 6	36	40 0	9	3 ?	8	8 8
6 9	64	144	54 1	154	58 3	1 0	45 4	58	? 0
9 1?	365	18?	50 0	[illegible]1	59 4	193	53 0	89	4 1
0 9	174	95	54 6	110	63 ?	91	5 3	43	[illegible]5 0
30 and over	104	68	65 4	5	[illegible] 1	61	58 6	40	38 4

[illegible]

Fig. 11[illegible]

types of polio virus develops are so varied that the probability of developing poliomyelitis a second time is in principle very small

If the statement that people living in a polio virus infected environment or subjects in contact with poliomyelitis patients polio virus secretors or poliomyelitis virus cultures have a lower anti body deficiency and an increased antibody titer is true this observation could make it necessary to take into account simultaneous dissemination of polio viruses when the effect of artificial immuni zation is evaluated The inoculation of a popula tion which has only infrequent and slight contact with polio viruses can lead merely to a shorter im munization than if the dissemination of the polio viruses brings with it more frequent contacts thereby leading to a stimulation effect In the type of case first mentioned (that of Berlin for exam ple) involving limited dissemination of virus several timely vaccinations during a person s life time as in the case of smallpox should be consid ered If polio protective vaccination is once intro duced continuation of such vaccination in future years will have to be planned not haphazard

Enteric Viruses Producing Disease Simulating Poliomyelitis

TUESDAY AFTERNOON, JULY 9, 1957

Chairman

PROF DR C H STUART HARRIS
Department of Medicine
Royal Hospital
Sheffield England

Speakers

ECHO VIRUSES

DR JOSEPH L MELNICK
College of Medicine
Baylor University
Houston

DR DAVID T KARZON
Department of Bacteriology and Immunology
University of Buffalo
Buffalo

DR HERBERT A WENNER
Department of Pediatrics
University of Kansas Medical Center
Kansas City Kan

COXSACKIE VIRUSES

DR GILBERT J DALLDORF
State of New York
Department of Health
Albany

DR MICHAEL L FURCOLOW
U S Public Health Service
Kansas City Kan

DR JAMES H S GEAR
South African Institute for Medical Research
Johannesburg

Discussants

Moderator PROF DR van CREVELD
Pediatric Clinic
University of Amsterdam
Amsterdam

DR GUILLERMO CONTRERAS
Instituto Bacteriologico
Santiago Chile

DR WILLIAM McD HAMMON
Graduate School of Public Health
University of Pittsburgh
Pittsburgh

DR TORSTEN JOHNSSON
State Bacteriological Laboratory
Department of Virus Research
Karolinska Institute
Stockholm

DR F O MacCALLUM
Central Public Health Laboratory
London

PROF DR E NIHOUL
Ruksuniversiteit-Ghent
Ghent

PROF JAROSLAV PROCHAZKA
Bulovka Hospital
Prague

DR B SIGURDSSON
Institute Experimental Pathology
Reykjavik

DR ARNE SVEDMYR
Virus Department
Central Bacteriological Laboratory
Stockholm

PROF DR J D VERLINDE
Netherlands Institute for Preventive Medicine
Leiden

The ECHO Viruses and Their Classification

DR JOSEPH L MELNICK

As soon as tissue-culture methods began to be applied to the isolation of viruses it became obvious that viruses other than poliomyelitis and the Coxsackie viruses existed in the human enteric tract It early became apparent that these agents could be isolated from normal children as well as from patients with the aseptic meningitis syndrome and that multiple antigenic types exist As more investigators entered the field more and more of these untypable agents came to be recognized especially in those laboratories using monkey kidney cultures Because the human diseases to which they belonged were unknown and because they failed to produce illness in laboratory animals these agents were called the orphan viruses

A working committee formed to facilitate the classification of these new agents found what it believed to be a more suitable name for these viruses and proposed that they be called the *enteric cytopathogenic human orphan* or ECHO viruses A co-operative study on the prototype strains then available (8 in our laboratory 5 in Dr Sabin s and 3 in Dr Hammon s) resulted in the differentiation of 13 antigenically distinct viruses None of the original 13 prototype viruses or the 6 or 7 new ones which have been identified subsequently had been found to multiply and produce disease in laboratory animals However there may be some need to qualify this aspect of the definition of ECHO viruses in view of some recent observations on which comment will be made later

The laboratory data required to establish a strain as a new prototype are shown in Table 72 Here *we can see the data required to establish the Tow strain* as the prototype strain of No 14 In addition to the failure of the Tow strain to be neutralized by the antisera shown these antisera being effective against the other known ECHO viruses including each of the previously known types a new serum had to be prepared which was specific for the virus in that the preimmunization serum was negative but the postimmunization serum contained antibodies for this type alone A more or less uniform technic was adopted A new prototype was established as one which at a test dose of 100 tissue-culture doses failed to be neutralized by 20 units of antibody against each of the previously known virus types conversely its serum at a concentration of 20 units failed to neutralize each of the other virus types Because ECHO mixtures may occur in nature the ECHO Virus Committee has recommended that all sera to new types be prepared with virus particles derived from a single clone obtained either from a plaque or from a terminal dilution culture.

Neutralization tests as shown in Table 72 were also conducted with paired serum specimens obtained from the patient from whom the virus was recovered In accordance with the

TABLE 72 NEUTRALIZATION TESTS ESTABLISHING TOW STRAIN AS ECHO TYPE 14

A SPECIFIC NEUTRALIZATION OF TOW STRAIN

SERA	TITERS AGAINST 100 TCD_{50} OF TOW VIRUS
Monkey No 8076	
Preinoculation serum	<10
Postinoculation with Tow strain	320
Patient Tow	
4th day after onset	<10
93rd day after onset	100

B TOW VIRUS (32 TO 100 TCD_{50}) NOT NEUTRALIZED BY

Antisera to poliovirus Types 1 2 3
Antisera to Coxsackie virus Types
A-1 2 3 4 5 6 7 8 9 10 1[illegible] 14
B-1 2 3 4 5
Antisera to ECHO virus Types
1 2 3 4 5 6 7 8 9 10 11 12 13

At a dilution of 1:10 the postimmunization serum failed to neutralize ECHO viruses 1 through 13

TABLE 73 SPECIFICITY OF ECHO TYPE 14 (TOW STRAIN) IN THE COMPLEMENT FIXATION REACTION

SERUM	COMPLEMENT FIXING ANTIBODY TITER AGAINST				
	ADENOVIRUS ANTIGEN	POLIO ANTIGENS			TOW VIRUS ANTIGEN*
		TYPE 1	TYPE 2	TYPE 3	
Tow monkeys					
No 8017					
Preinoculation serum	<8	<8	<8	<8	<8
Postinoculation serum	<8	<8	<8	<8	*1 8*
No 8076					
Preinoculation serum	<8	<8	<8	<8	<8
Postinoculation serum	<8	<8	<8	<8	*128*
Adenovirus antiserum	> *128*	<8	<8	<8	<16
Polio virus antiserum					
Type 1	<30	*240*	<30	<30	<30
Type 2	<30	<30	*120*	<30	<30
Type 3	<30	<30	<30	*240*	<30

Tissu -culture flu d at a diluti n of 1 2 hich conta d 8 units of antigen

principles of classical immunology the patient developed neutralizing antibodies during his convalescence

Complement fixing antibodies also appeared in monkeys inoculated with the Tow strain of virus (Fig 118) The CF tests listed in Table 73 indicate that the antibodies were specific for the new type They fail to cross with the adenoviruses or with the polio virus antigens

I mention here that all is not quite as simple and clear as it might seem For example as shown in Table 74 certain ECHO viruses cross-

TABLE 74 RELATIONSHIPS AMONG ECHO VIRUSES 6 6 AND 6

VIRUS (100 TCD)	SERUM TESTED			
	TYPE 6 RH 7819	TYPE 6 CHIMP 45	TYPE 6 RH 8922	TYPE 6 RH 8?89
	A SERUM TITER			
Type 6 (D Amori)	320	64 000	600	60 000
Type 6 (DiMeo)	10	150	300	2 400
Type 6 (Burgess)	0	30	20	2 100
	B NEUTRALIZATION RATIO †			
Type 6 (D Amori)	*1 0*	*1 0*	2 0	28 6
Type 6 (DiMeo)	0 03	0 002	*1 0*	1 1
Type 6 (Burgess)	0	0 0005	0 07	*1 0*

0—titer of less than 10

† N utralizat on R tio = (T te ag n t hete logo iru) / (Titer ag n t homologo us)

(H m l ou rum arbitrar ly given alue of 1 0)

react but chiefly in one direction Strains which have a broader antigenic composition than the prototype strains have been classified provisionally as prime strains Thus some strains have been recognized which are related to but are not identical with the prototype strain of Type 6 These 6 strains produce antisera which neutralize ECHO-6 prototype strain the 6 strains are not neutralized by Type 6 antisera unless the sera have a high Type 6 antibody titer as shown by the serum of the chimpanzee in the second column which partially neutralizes Type 6 Type 6 strains seem to have even broader antigenic overlap with the prototype ECHO-6 strain in that their antisera manifest a higher titer against ECHO 6 than against the homologous virus used to immunize the monkey Antigenic variance exists among other types also notably among Types 5 9 and 11

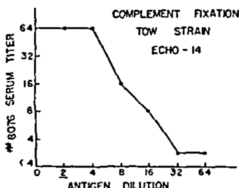
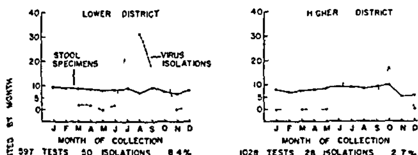


Fig 118 Complement fixation box titration of Tow antiserum from monkey No 8076 against Tow antigen The tissue-culture fluid was used at a dilution of 1 2 which contained 8 units of antigen

CHARLESTON



PHOENIX

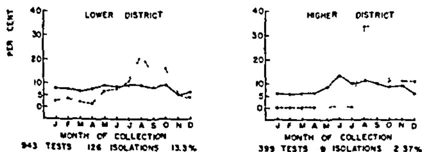


Fig 119 Stool collections and enteric virus isolations cumulative for each month over a 3 year period (1951 1953) Specimens were obtained from healthy children in contrasting socioeconomic districts of Charleston W Va., and Phoenix Ariz., during a nonepidemic period

Recent studies by members of the Committee on the ECHO Viruses have shown that the prototype ECHO 1 and ECHO 13 strains may have to be reconsidered as belonging to distinct types It had been found that the ECHO 1 typing serum which we had originally prepared when the virus was first identified failed to neutralize a new prototype strain isolated in Dr Hammon s laboratory This particular strain had been designated as ECHO 13 New antisera against ECHO 1 have been prepared and they have been found to neutralize not only ECHO 1 but also ECHO 13 virus conversely new ECHO 13 antisera prepared with later passages of the virus now neutralize ECHO 1 virus The full details of these experiments which will be published as a Committee Report suggest that ECHO 13 is a mixture of two viruses ECHO 1 plus an antigenically distinct ECHO 13 or perhaps that ECHO 1 shows a prime relationship to ECHO 13

I turn now to the epidemiology of the ECHO viruses Several laboratories in many countries are conducting studies on the viral flora of the human enteric tract We in conjunction with the Epidemiology Branch of the Communicable Disease Center have recently completed a longitudinal study of the endemic occurrence of enteric viruses among several hundred normal households in Charleston West Virginia and Phoenix Arizona Some relevant findings indicate what may be learned from such studies. Approximately equal numbers of stools were collected each month from normal children living under contrasting environments However almost all of the virus isolations were made in the summer and the fall except from the lower socioeconomic district of Phoenix (Fig 119) where viruses were recovered throughout a larger part of the year Figure 120 illustrates the repeatable seasonal incidence of enteric virus excretion among normal children

The frequency of virus excretion in the lower socioeconomic district of each city was 3 to 6

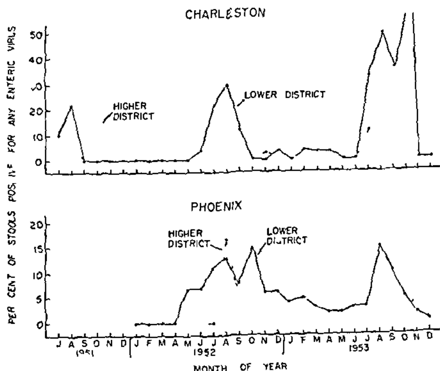


Fig 120 Data of Figure 119 arranged to show the per cent of stool specimens yielding enteric viruses by mouth through the study period in higher and lower socioeconomic districts

TABLE 75 PER CENT DISTRIBUTION OF ENTERIC VIRUSES ISOLATED FROM HEALTHY CHILDREN IN POPULATIONS OF CONTRASTING SOCIOECONOMIC LEVEL DURING A NONEPIDEMIC PERIOD (1951 53)

Population Group	Number of Specimens Tested	Per Cent Yielding Viruses: Polio Viruses	Coxsackie Viruses	ECHO Viruses	All Enteric Viruses
Charleston W Va					
Lower	597	2 3	2 3	3 7	8 4
Upper	1 028	0 5	1 5	0 8	2 7
Phoenix Ariz					
Lower	943	3 0	2 0	8 3	13 3
Upper	399	1 0	1 0	0 3	2 3
Total					
Lower	1 540	2 8	2 1	6 6	11 4
Upper	1 427	0 [illegible]	1 3	0 6	2 6

times as great as in the middle to upper middle class districts with good environmental sanitation If the combined figures are examined—here I call attention to the bottom two rows of Table 75—of 1 540 specimens tested in the lower districts 176 or 11 per cent were positive whereas in the upper districts only 2 6 per cent —37 of 1 427 specimens—yielded virus Among the 213 viruses isolated in monkey kidney cultures 52 per cent were ECHO viruses 24 per cent were polio viruses and 24 per cent were Coxsackie viruses

ASSOCIATION OF ECHO VIRUSES WITH ASEPTIC MENINGITIS

Although some ECHO viruses have been recovered from patients with aseptic meningitis clinically indistinguishable from nonparalytic polio the epidemiologic criteria regarding all of the ECHO viruses as etiologic agents of this syndrome have not yet been met However certain members have been associated with aseptic meningitis outbreaks and certain types notably Types 5 6 9 14 and perhaps others have been isolated from the cerebrospinal fluid of aseptic meningitis patients in the laboratories of Rhodes Karzon DeSomer as well as others

The experience with aseptic meningitis cases in Connecticut during the past 2 years is summarized in Table 76 It can be seen that ECHO Type 6 strains and the Coxsackie viruses of Types B 2 B 3 B-4 and A 9 were as frequent contributing factors to aseptic meningitis as were polio viruses Over the 2 year period

TABLE 76 DISTRIBUTION OF ENTERIC VIRUS ISOLATIONS FROM ASEPTIC MENINGITIS CASES IN CONNECTICUT DURING 1955 AND 1956*

Virus Isolated	Number of Isolations: From 13[illegible] Cases Studied in 195[illegible]	From 8[illegible] Cases Studied in 1956	From Total of 2[illegible]1 Cases
Polio virus	[illegible]8	5	3[illegible]
ECHO 6	[illegible]	11	3[illegible]
ECHO 14		0	2
Coxsackie A 9	1	8	9
Coxsackie B [illegible] B-3 B 4	15	4	19
Unidentified virus		4	6
Total virus isolation	[illegible]	[illegible]	100

* [illegible] 1956 [illegible]

TABLE 77 ANTIBODY TITERS TO ECHO-6 VIRUS AND THE POLIO VIRUSES OBSERVED IN PAIRED SERA OF ASEPTIC MENINGITIS PATIENTS EXCRETING ECHO-6 VIRUS

Patient No	Serum	ECHO 6 Neutralizing Antibody Titer	Polio Virus Antibody Titer: Neutralizing 1	2	3	Complement Fixing 1	2	3
1	Acute	0	0	0	64	0	0	0
	Conv	100	0	0	64	0	0	0
2	Acute	0	256	0	0	0	0	0
	Conv	100	256	0	0	0	0	0
3*	Acute	0	256	1 024	1 024	0	0	0
	Conv	50	256	1 024	1 024	0	0	0
4*	Acute	10	64	1 024	16	0	4	0
	Conv	100	64	1 024	16	0	4	0

Patients 3 and 4 had been vaccinated against poliomyelitis some months before they became ill with aseptic meningitis Their vaccination rather than natural infection probably accounts for the high level of neutralizing antibodies to all 3 types of polio virus and the lack of CF antibodies children in Connecticut at the ages of these patients (6 and 8 years) rarely have had natural infections with all 3 types

which is summarized in the last column on the table there were 32 polio virus isolations 32 isolations of ECHO 6 and 28 isolations of the Coxsackie viruses listed from the total of 221 aseptic meningitis cases studied Considering only those cases from which any virus was isolated polio viruses and ECHO 6 each accounted for approximately 30 per cent of the total isolations with the Coxsackie viruses accounting for about 27 per cent Results of serologic tests on 4 patients excreting ECHO 6 virus are shown in Table 77

A number of strains of a single type recently isolated by workers in England Belgium Holland Denmark Germany Italy and Canada are worthy of special comment A number of isolations were made by McLean from an epidemic of aseptic meningitis in Bourne England We had the privilege of studying this material and were able to identify the virus as ECHO 9 However I would like to call attention to the incomplete crossings obtained with the Bourne strain from England for example as shown in Table 78 in the same test using the same lot of tissue-culture tubes two established strains of ECHO 9 (Hill and Quigley) along with the Bourne strain from England were used to titrate the neutralizing capacity of their respective sera While

TABLE 78 CROSS RELATIONSHIPS BETWEEN ESTABLISHED ECHO 9 STRAINS AND THE BOURNE STRAIN

Virus	Serum Titer Against 100–320 TCD_{50} of Virus: Hill Antiserum	Quigley Antiserum	Bourne Antiserum	Neutralization Ratio*: Hill Antiserum	Quigley Antiserum	Bourne Antiserum
Hill	15 600	2 500	900	*1.00*	1.67	0.11
Quigley	15 600	1 500	3 400	1.00	*1.00*	0.40
Bourne MK_6†	5 400	220	8 500	0.35	0.15	*1.00*
Bourne MK_4 m ‡	7 800			0.50		

* Neutralization Ratio = Titer against heterologous virus / Titer against homologous virus

† MK_6—Virus used after 6 passages in monkey kidney

‡ $MK_6\ m_2$—Virus used after 6 passages in monkey kidney plus 2 passages in infant m

there was complete crossing between the Hill and the Quigley strains both of these strains having been isolated in the United States the Bourne strain from England although antigenically related had a distinctive antigenic character It was poorly neutralized by the Quigley antiserum and in turn as shown in the last column of Table 78 the Bourne antiserum gave a tenfold higher titer against the homologous strain than it did against the Hill prototype strain

The Bourne virus was associated with a sharp epidemic of frank aseptic meningitis and subsequently with two outbreaks characterized by milder symptoms Since ECHO-9 was the only virus isolated from the patients studied it seems reasonable to infer that this virus caused each outbreak Rising titers of neutralizing antibody to current strains of ECHO 9 further strengthen this view From these results it would appear that ECHO 9 virus as well as ECHO-6 must be added to the list of agents which may cause the aseptic meningitis syndrome Support of this view has recently come from several sources A virus isolated in monkey kidney cells by Dr DeSomer in Louvain from the spinal fluid of an aseptic meningitis patient and sent to New Haven for typing also turned out to be an ECHO 9 virus DeSomer isolated the same virus from the spinal fluids of 7 cases of aseptic meningitis and from 76 fecal specimens Serologic studies showed eightfold or greater antibody increases during convalescence Tyrrell and Snell in England have recently described an epidemic disease characterized by rash and aseptic meningitis some cases of which were similar to the disease observed earlier in babies in East London 3 years ago ECHO 9 viruses were isolated in tissue culture by both groups of workers The same virus was isolated repeatedly from cerebrospinal fluids as well as from throat washings and feces during the 1956 aseptic meningitis epidemic which occurred throughout Europe notably in England Denmark Belgium Holland Germany and also in Italy

In addition to the association of ECHO 9 with outbreaks of aseptic meningitis and its recovery from the cerebrospinal fluid one other important fact has come out of these studies namely that certain strains of ECHO 9 virus after several passages in monkey kidney tissue produce paralysis and myositis in infant mice typical of the Group A Coxsackie viruses The mouse virus has a high titer in tissue culture and in mice ECHO 9 prototype serum when titrated in tissue culture against the Quigley strain the prototype Hill strain the Quigley strain the Bourne strain from England either in monkey kidney passage or in mouse passage gave similar titers As Table 79 shows at a dilution of 1 to 100 the serum neutralized 1 000 000 tissue-culture doses of virus grown in mice for 7 passages and then used as seed for the neutralization test That the mouse pathogenic virus was identical with the virus grown in tissue culture was shown by neutralization tests in mice (Table 79 second column) The prototype Hill serum also at a dilution of 1 to 100 neutralized over 10 000 mouse paralytic doses of virus The question

TABLE 79 NEUTRALIZATION TEST ON SERA TAKEN DURING THE BOURNE EPIDEMIC OCTOBER 1955

Patient No	Serum Titer Against Mouse Pathogenic Bourne Strain: Acute	Mouse Pathogenic Bourne Strain: Convalescent	Mouse Apathogenic Quigley Strain: Acute	Mouse Apathogenic Quigley Strain: Convalescent
1	0	>100	0	50
3	0	50	0	100
4	0	50		
5	10	?50		
6	50	> 50		
	50	<50		

0 indicates less than 10

TABLE 77 ANTIBODY TITERS TO ECHO-6 VIRUS AND THE POLIO VIRUSES OBSERVED IN PAIRED SERA OF ASEPTIC MENINGITIS PATIENTS EXCRETING ECHO-6 VIRUS

Patient No	Serum	ECHO 6 Neutralizing Antibody Titer	Polio Virus Antibody Titer: Neutralizing 1	Neutralizing 2	Neutralizing 3	Complement fixing 1	Complement fixing 2	Complement fixing 3
1	Acute	0	0	0	64	0	0	0
	Conv	100	0	0	64	0	0	0
2	Acute	0	256	0	0	0	0	0
	Conv	100	256	0	0	0	0	0
3*	Acute	0	256	1 024	1 024	0	0	0
	Conv	50	256	1 024	1 024	0	0	0
4*	Acute	10	64	1 024	16	0	4	0
	Conv	100	64	1 024	16	0	4	0

Patients 3 and 4 had been vaccinated against poliomyelitis some months before they became ill with aseptic meningitis. Their vaccination rather than natural infection probably accounts for the high level of neutralizing antibodies to all 3 types of polio virus and the lack of CF antibodies; children in Connecticut at the ages of these patients (6 and 8 years) rarely have had natural infections with all 3 types.

which is summarized in the last column on the table there were 32 polio virus isolations 32 isolations of ECHO-6 and 28 isolations of the Coxsackie viruses listed from the total of 271 aseptic meningitis cases studied. Considering only those cases from which any virus was isolated polio viruses and ECHO 6 each accounted for approximately 30 per cent of the total isolations with the Coxsackie viruses accounting for about 27 per cent. Results of serologic tests on 4 patients excreting ECHO 6 virus are shown in Table 77.

A number of strains of a single type recently isolated by workers in England Belgium Holland Denmark Germany Italy and Canada are worthy of special comment. A number of isolations were made by McLean from an epidemic of aseptic meningitis in Bourne England. We had the privilege of studying this material and were able to identify the virus as ECHO 9. However I would like to call attention to the incomplete crossings obtained with the Bourne strain from England for example as shown in Table 78 in the same test using the same lot of tissue-culture tubes two established strains of ECHO 9 (Hill and Quigley) along with the Bourne strain *from England* were used to titrate the neutralizing capacity of their respective sera. While

TABLE 78 CROSS RELATIONSHIPS BETWEEN ESTABLISHED ECHO 9 STRAINS AND THE BOURNE STRAIN

Virus	Serum Titer Against 100–320 TCD of Virus: Hill Antiserum	Quigley Antiserum	Bourne Antiserum	Neutralization Ratio: Hill Antiserum	Quigley Antiserum	Bourne Antiserum
Hill	15 600	2 500	900	*1.00*	1.67	0.11
Quigley	15 600	1 500	3 400	1.00	*1.00*	0.40
Bourne MK_6†	5 400	220	8 500	0.35	0.15	*1.00*
Bourne MK_6m_2‡	7 800			0.50		

$$\text{Neutralization Ratio} = \frac{\text{Titer against heterologous virus}}{\text{Titer against homologous virus}}$$

† MK_6—Virus used after 6 passages in monkey kidney

‡ MK_6m_2—Virus used after 6 passages in monkey kidney plus 2 passages in infant mice

there was complete crossing between the Hill and the Quigley strains both of these strains having been isolated in the United States the Bourne strain from England although antigenically related had a distinctive antigenic character It was poorly neutralized by the Quigley antiserum and in turn as shown in the last column of Table 78 the Bourne antiserum gave a tenfold higher titer against the homologous strain than it did against the Hill prototype strain

The Bourne virus was associated with a sharp epidemic of frank aseptic meningitis and subsequently with two outbreaks characterized by milder symptoms Since ECHO 9 was the only virus isolated from the patients studied it seems reasonable to infer that this virus caused each outbreak Rising titers of neutralizing antibody to current strains of ECHO 9 further strengthen this view From these results it would appear that ECHO 9 virus as well as ECHO-6 must be added to the list of agents which may cause the aseptic meningitis syndrome Support of this view has recently come from several sources A virus isolated in monkey kidney cells by Dr DeSomer in Louvain from the spinal fluid of an aseptic meningitis patient and sent to New Haven for typing also turned out to be an ECHO 9 virus DeSomer isolated the same virus from the spinal fluids of 7 cases of aseptic meningitis and from 76 fecal specimens Serologic studies showed eightfold or greater antibody increases during convalescence Tyrrell and Snell in England have recently described an epidemic disease characterized by rash and aseptic meningitis some cases of which were similar to the disease observed earlier in babies in East London 3 years ago ECHO 9 viruses were isolated in tissue culture by both groups of workers The same virus was isolated repeatedly from cerebrospinal fluids as well as from throat washings and feces during the 1956 aseptic meningitis epidemic which occurred throughout Europe notably in England Denmark Belgium Holland Germany and also in Italy

In addition to the association of ECHO 9 with outbreaks of aseptic meningitis and its recovery from the cerebrospinal fluid one other important fact has come out of these studies namely that certain strains of ECHO 9 virus after several passages in monkey kidney tissue produce paralysis and myositis in infant mice typical of the Group A Coxsackie viruses The mouse virus has a high titer in tissue culture and in mice ECHO 9 prototype serum when titrated in tissue culture against the prototype Hill strain the Quigley strain the Bourne strain from England either in monkey kidney passage or in mouse passage gave similar titers As Table 79 shows at a dilution of 1 to 100 the serum neutralized 1 000 000 tissue-culture doses of virus grown in mice for 2 passages and then used as seed for the neutralization test That the mouse pathogenic virus was identical with the virus grown in tissue culture was shown by neutralization tests in mice (Table 79 second column) The prototype Hill serum also at a dilution of 1 to 100 neutralized over 10 000 mouse paralytic doses of virus The question

TABLE 79 NEUTRALIZATION TESTS ON SERA TAKEN DURING THE BOURNE EPIDEMIC OCTOBER 1955

PATIENT No	SERUM TITER AGAINST			
	MOUSE PATHOGENIC BOURNE STRAIN		MOUSE APATHOGENIC QUIGLEY STRAIN	
	ACUTE	CONVALESCENT	ACUTE	CONVALESCENT
1	0*	>100	0	250
2	0	50	0	100
3	0	250		
4	10	250		
5	50	>250		
6	50	250		

0 indicates l th 10

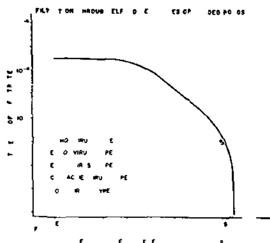


Fig 121 Correlation of pore size of membrane and filtrability of ECHO virus Types 1 2 and 3 Coxsackie virus Type A 9 and polio virus Type 1 all grown in monkey tissue culture

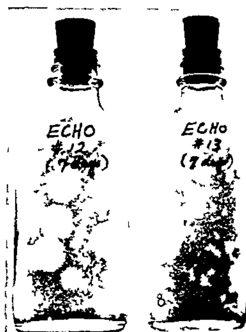


Fig 123 Representative plaques of the two groups of ECHO viruses (*Left*) Type B—ECHO 12 (*Right*) Type A—ECHO 13

must be raised at this time—although it cannot be answered as yet—as to whether ECHO-9 really belongs with the ECHO viruses or with the Coxsackie viruses If many perhaps the majority of strains remain nonpathogenic for *mice even after repeated attempts with virus* carried serially in tissue culture then this raises a difficult problem in classification If the scheme of classification rests on the host strains of virus then the host strains of the natural virus before *laboratory manipulation* might well be the criterion of choice It certainly makes the test for host strains easier to carry out than one which requires as a preliminary to the test an indefinite number of tissue-culture passages This

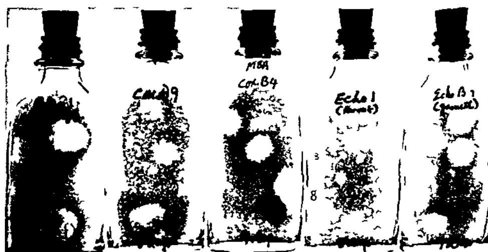


Fig 122 Rhesus bottle cultures of polio virus Type 1 (Mahoney) Coxsackie virus Types A 9 (Grigg) and B-4 (Texas 13) and ECHO virus Types 1 (Farouk) and 7 (Garnett)

leads to another problem in that we have isolated a number of Coxsackie A 9 and B strains which failed to take in infant mice unless they are first passaged in tissue culture Obviously we are only at the beginning in the study of these agents and questions of the classification of the Cox ECHO viruses will become resolved only as more data become available

PROPERTIES OF THE ECHO VIRUSES

Ultrafiltration experiments carried out with Allister Macrae have shown that ECHO viruses 1 2 and 3 have the same size as the Coxsackie and the polio viruses These viruses pass 38 mu gradocol membranes but are held back by membranes having an average pore diameter of 30 millimicrons In experiments carried out under the same conditions Coxsackie A 9 and polio virus Type 1 had the same filtration properties (Fig 121) Work on the size of these viruses has been continued in our laboratory using the method of ionizing radiation in collaboration with Dr Pollard From studies of the rate of viral inactivation produced by irradiation with high energy electrons deuterons or alpha particles the ECHO viruses studied again proved to be similar in size to the polio viruses It is of interest that as in the polio virus particle the infectious unit has at least twice the diameter of the complement fixing antigen as calculated from its more rapid rate of inactivation

The growth of animal viruses in cells under agar produces areas of necrosis as first shown by Dr Dulbecco and the morphology of these virus colonies or plaques as they are called is an aid in the characterization of these agents (Fig 122) In rhesus cultures plaques of ECHO viruses usually appear a few days later than polio virus plaques Plaques of certain ECHO virus types are irregular in shape and often their boundaries are diffuse In contrast to these viruses provisionally called Group A in our laboratory are the ECHO viruses in Group B Except for some strains of Type 8 these viruses resemble polio viruses in their plaque morphology As shown in Figure 123 in Type 12—a member of this particular group —the plaques are circular with clear centers and sharp boundaries indistinguishable from those of poliomyelitis viruses The Group B ECHO viruses grow rapidly the plaques being 1 cm or over in diameter 1 week after seeding In contrast the ECHO viruses of Group A—Type 13 in Figure 123—grow more slowly and as a rule are only ½ cm in diameter when measured 10 days after seeding In natural mixed infections small amounts of polio virus particles were isolated easily and quickly by passing progeny from the larger circular plaques Passing the progeny of the small plaques gave pure cultures of the ECHO virus (Fig 124)

Important in the characterization of a virus is the determination of its host range A sum

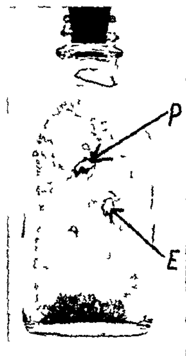


Fig 124 Bottle culture inoculated with a stool specimen containing a mixture of virus particles Passage of the large plaque yielded a polio virus and of the small plaque an ECHO virus Arrows indicate sites at which agar was picked from the polio virus plaque (P) and from the ECHO plaque (E) It is advantageous to pick from a site at the boundary of a plaque where the most recent virus proliferation has occurred Because of thermal inactivation the center of a large plaque may not contain viable virus

TABLE 80 SUSCEPTIBILITY RATIO OF REPRESENTATIVE MONKEY SPECIES TO POLIOMYELITIS COXSACKIE AND ECHO VIRUSES*

MONKEY	POLIO VIRUS TYPE 2†	COXSACKIE VIRUS TYPE A 9	COXSACKIE VIRUS TYPE B 1‡	ECHO VIRUS TYPE 1§	ECHO VIRUS TYPE 7
Patas (*Erythrocebus patas*)	2 4	0 00000001	2 0	0 000000001	1 [illegible]
Spot nose (*Cercopithecus nictitans buttikoferi*)	1 0	0 00000001		0 000000001	1 9
Green (*Cercopithecus aethiops sabaeus*)	1 9	1 7		2 4	2 3
Sooty mangabey (*Cercocebus fuliginosus*)	1 1	1 7		1 1	1 2
Olive baboon (*Papio doguera*)	1 3	1 3		1 1	1 3
Rhesus (*Macaca mulatta*)	1 0	1 0	1 0	1 0	1 0

$$\text{Susceptibility Ratio} = \frac{\text{Average plaque count in cultures of species tested}}{\text{Average plaque count in rhesus cultures}}$$

† Polio virus Types 1 and 3 resemble Type 2
‡ Coxsackie viruses B 2—B 5 resemble B 1
§ ECHO virus Types 3 4 6 9 11 13 and 14 resemble Type 1

mary of work on the susceptibility of monkey kidney cells to polio Coxsackie and ECHO viruses by the plaque method is given in Table 80

Cells from a number of African species were similar to those of rhesus in their susceptibilities in contrast with those from Erythrocebus patas the African red grass monkey As shown in the first line of the table patas cells were found to be twice as susceptible as rhesus cells to polio virus and about equal to rhesus in susceptibility to the Group B ECHO viruses and Group B Coxsackie viruses In contrast Coxsackie A9 and the Group A ECHO viruses failed to form plaques in patas cultures a property which has proved useful in identifying new viruses At

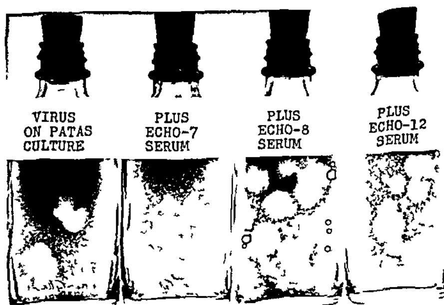


FIG 125 Virus identification by cell susceptibility and plaque morphology Unknown strain produced large round plaques on patas cultures as shown in bottle on the left Neutralization occurred with ECHO 7 antiserum (second bottle from left) but not with antiserum against ECHO 8 or ECHO 12

tually the only virus that we know which produces round plaques on rhesus and no plaques at all on patas is A 9 and in every case where this has turned up we have been able to identify the virus presumptively on this property

Recently more than 200 strains of ECHO viruses were studied using the technics of plaque morphology and cell susceptibility in patas and rhesus cultures All patas negative strains produced small irregular shaped plaques in rhesus bottles and all patas positive strains produced larger more circular plaques in cells of both species Neutralization tests thus far indicate that the patas-positive strains were neutralized by ECHO sera against Types 7 8 or 12 the so-called Group B ECHO viruses A neutralization test for a newly isolated strain of Type 7 is shown in Figure 125 The control bottle shows plaques in the presence of Type 7 there are no plaques in the bottle in the presence of 8 and 12

A classification of the ECHO viruses into Groups A and B as shown in Table 81 is supported by these studies on host cell susceptibility Group A produce small irregular shaped plaques on rhesus but are negative on patas cultures Group B—consisting of Types 7 8 and 12—are positive on patas cultures except for certain strains of Type 8 which produce smaller and more irregular plaques While the prototype strains of 2 3 5 and 6 have not produced typical plaques they were found to be cytopathogenic for rhesus but not for patas cells in tube cultures and are considered to be members of Group A So far the viruses associated with aseptic meningitis cases and those actually isolated from the spinal fluid of such patients have been members of Group A

THE EXPERIMENTAL INFECTION OF PRIMATES WITH THE ECHO VIRUSES

To obtain a better understanding of human viral infections it has proved useful in the past to study the experimental infection in the highest laboratory primate available namely the chimpanzee Infection of these anthropoids with ECHO viruses follows the pattern established with the other enteric viruses namely poliomyelitis and Coxsackie No apparent illness occurred among the chimpanzees but infection was readily demonstrated by the presence and persistence of virus in the throat and in the feces It is noteworthy that Type 6 virus was found in the throat as long as it was found in the intestines and Type 4 even longer in the throat than in the intestines

In the chimpanzees fed Type 4 virus persisted in the throat for 10 to 35 days but in the feces of these animals only from 3 to 10 days respectively This recalls a similar observation with the Coxsackie viruses which was limited to Type B 2 in which carriage of virus in the chimpanzee throat equaled that in the lower alimentary tract Unlike polio virus which readily finds its way from the skin to the gut in the chimpanzee the ECHO viruses studied were not readily excreted after parenteral infection In two animals infected with Type 4 virus was found briefly in the alimentary tract but this was not followed by long term carriage

As shown in Figure 126 the chimpanzee developed neutralizing antibodies with peak titers being reached within 2 to 4 weeks Data for 2 animals are shown in Figure 127 Antibodies persisted at their maximum level for more than 6 months and were not influenced by subsequent exposure to Type 4 virus The anti

TABLE 81 PROVISIONAL GROUPING OF ECHO VIRUSES*

GROUP	ECHO TYPES	GROWTH IN RHESUS CULTURES	GROWTH IN PATAS CULTURES
A†	1 *3* *4* *6* *9* 11 13 *14* 15 *16*	Plaques irregular shaped small	Negative
B	7 8 12	Plaques circular large‡	Positive

* Types in italic h[illegible] been associated with outbreaks of aseptic m[illegible] [illegible] isolated from the cerebrospinal fluid of aseptic meningitic cases

† Types 2 5 6 and the prototype strain of Type 3 (M[illegible]) which have not thus far produced plaques in the [illegible] culture [illegible] also do not produce [illegible] but are able to produce cytopathogenic effect in patas tube cultures

‡ Some Type 8 strains produce smaller and more irregular plaques



Fig 129 Plaques of ECMO virus strain 8 000 on monkey kidney monolayers (See text for details)

teric viruses for a number of different animal species

Enteric cytopathogenic monkey orphan or ECMO viruses are included among the simian viruses of Hull and his colleagues ECMO viruses—the monkey agents—have also been recovered by Cheever in Pittsburgh and by Riordan in New Haven A co-operative program among Hull s Cheever s and our laboratory is now under way in an attempt to classify the numerous agents which have been isolated In one series of 21 monkeys studied in New Haven 25 ECMO viruses have been recovered Some of these viruses were excreted for a period of weeks from the time the first isolation was made It is noteworthy that among the viruses isolated from the monkey enteric tract there have been a number of adenoviruses Our own experience in this regard was that 7 of the 25 monkey viruses isolated turned out to be adeno viruses

Some ECMO viruses have produced plaques resembling those of poliomyelitis or Coxsackie viruses described earlier others have produced plaques similar to those of the Group A ECHO viruses Examples of ECMO plaques are illustrated in Figure 129 in which strain 8 000 is shown 5 days after plating at concentrations of 10^{-3} and 10^{-4} At least 60 plaques are present at the higher concentration and 9 at the lower concentration This particular strain is one that is antigenically related to Hull s SV 16 Its plaques had clear boundaries but were characterized by the presence of islets of apparently healthy cells at the periphery of the plaque These are evident as the dark spots in the plaques shown in the illustration

SUMMARY

This paper has reviewed the developments in a field opened up by the introduction of new tissue-culture methods into virology From the time when an isolation of a new enteric virus was a rare finding often out of line with preconceived notions virologists are now at the stage of attempting to evaluate the significance of at least 20 new enteric viruses assembled together under the banner of ECHO viruses Although divided into 20 types their classification is complicated by the fact that subtypes exist which have antigenic moieties different from the prototype strains This situation is reminiscent of that which exists among the influenza viruses

Although newly recognized the ECHO viruses appear to be among the most common and widespread parasites of man Yet the only illness with which any of these viruses have been associated is aseptic meningitis and thus they often confuse the picture of nonparalytic poliomyelitis Among the ECHO viruses only members of Group A have been isolated from the cerebrospinal fluid or have been otherwise associated with aseptic meningitis It may well be that certain ECHO types are not pathogenic for the human species and that their isolation from man has no more significance than the isolation of saprophytic bacteria or certain members of the Coxsackie group which have not yet found their disease And yet because we know so little about the factors which might lead a virus toward an increase in virulence and the production of serious illness the continued study of the ECHO viruses would seem to be a highly desirable pursuit

BIBLIOGRAPHY

Boissard G P B Stoke L J Macrae A D and MacCallum F O Personal communication

Cheever F S Discussion of paper by Hull and Minner Ann New York Acad Sc 67 427 1957

Committee on ECHO Viruses National Foundation for Infantile Paralysis Enteric cytopathogenic human orphan (ECHO) viruses Science 122 1187 1955

Crawford M Macrae A D and O Reilly J N An unusual illness in young children associated with an enteric virus Arch Dis Childhood 31 182 1956

Davis D C and Melnick J L Association of ECHO virus type 6 with aseptic meningitis Proc Soc Exper Biol & Med 92 839 1956 and unpublished work

DeSomer P Personal communication

Dulbecco R Production of plaques in monolayer tissue cultures by single particles of an animal virus Proc Nat Acad Sc 38 747 1952

Fox J P Gelfand H M LeBlanc D R and Conwell D P A continuing study of the acquisition of natural immunity to poliomyelitis in representative Louisiana households Am J Pub Health 46 283 1956

Hammon W McD Ludwig E H Pavia R A McCloskey L W and Sather G E Problems raised by certain ECHO viruses in the attempted laboratory detection of polio myelitis virus infection Ann New York Acad Sc 67 304 1957

Hammon W McD Sabin A Wenner H A Ormsbee R A and Melnick J L Reports to be published

Honig E I Melnick J L Isacson P and Parr R An epidemiological study of enteric virus infections Poliomyelitis Coxsackie and orphan (ECHO) viruses isolated from normal children in two socio-economic groups J Exper Med 103 247 1956

Hsiung G D and Melnick J L Comparative susceptibility of kidney cells from different monkey species to enteric viruses (poliomyelitis Coxsackie and ECHO groups) J Immunol 78 137 1957

——— Morphologic characteristics of plaques produced on monkey kidney monolayer cultures by enteric viruses (poliomyelitis Coxsackie and ECHO groups) J Immunol 78 128 1957

——— Plaque formation with poliomyelitis Coxsackie and orphan (ECHO) viruses in bottle cultures of monkey epithelial cells Virology 1 533 1955

Hull R N Minner J R and Smith J W New viral agents recovered from tissue cultures of monkey kidney cells Am J Hyg 63 204 1956

Isacson P and Melnick J L Manuscript in preparation 1957

Itoh H and Melnick J L Infection of chimpanzees with ECHO viruses J Exper Med 105 677 1957

Klein M Personal communication

Kunin C M and Minuse E The isolation in tissue culture and chick embryo of filtrable agents from healthy dairy cattle J Immunol 80 1 1958

McLean D M and Melnick J L Association of a mouse pathogenic strain of ECHO virus type 9 with aseptic meningitis Proc Soc Exper Biol & Med 94 656 1957

Melnick J L Application of tissue culture methods to epidemiological studies of poliomyelitis Am J Pub Health 44 571 1954

——— Problems associated with viral identification and classification in 1956 Ann New York Acad Sc 67 363 1957

Melnick J L and Agren K Poliomyelitis and Coxsackie viruses isolated from normal infants in Egypt Proc Soc Exper Biol & Med 81 621 1952

Melnick J L Riordan J T Curnen E C and Macrae A D Tissue-culture pathogenic agents other than poliomyelitis virus isolated from cases of suspected poliomyelitis Fed Proc 12 454 1953

Melnick J L Walton M Isacson P and Cardwell W Environmental studies of endemic enteric virus infections I Community sero immune patterns and poliovirus infection rates Am J Hyg 65 1 1957

Meyer H M Jr Personal communication

Moscovici C Ginevri A and Mazzaracchio V Isolamento su coltura di tessuti di un virus da enterita suina Zooprofilassi July 7 1956

Ormsbee R A and Melnick J L J Immunol 79 384 1957

Proc of Conference on Orphan Viruses May 1955 Distributed to members of the Conference and on file at the offices of the National Foundation for Infantile Paralysis

Ramos Alvarez M and Sabin A B Intestinal viral flora of healthy children demonstrable by monkey kidney culture Am J Pub Health 46 295 1956

Riordan J T Ledinko N and Melnick J L

Multiplication of poliomyelitis viruses in tissue cultures of monkey testes Am J Hyg 55 339 1952
Robbins F C Enders J F Weller T H and Florentino G L Studies on the cultivation of poliomyelitis viruses in tissue culture V The direct isolation and serologic identification of virus strains in tissue culture from patients with nonparalytic and paralytic poliomyelitis Am J Hyg 54 286 1951
Tyrrell D A J and Snell B Recovery of a virus from cases of an epidemic exanthem associated with meningitis Lancet 271 1028 1956

Outbreaks of Aseptic Meningitis Caused by Type 6 ECHO Virus

DR DAVID T KARZON

An outbreak of aseptic meningitis caused by ECHO-6 in western New York State during the summer of 1955 was studied. Aseptic meningitis associated with ECHO-6 virus has also been observed in northeastern United States by Kibrick, Melendes and Enders and by Davis and Melnick in 1954 and 1955. In the present study 156 hospitalized aseptic meningitis patients and 485 household associates were examined (Table 82). ECHO-6 virus was recovered in monolayer monkey kidney tissue culture from stools, throat swabs and spinal fluids of 68 per cent of the patients as seen in the column on the right, while polio virus was recovered from 4 per cent and other cytopathogenic agents from 6 per cent. ECHO-6 was recovered from the spinal fluid of 8 patients. Neutralizing antibody to ECHO 6 appeared by the fifth day of illness and persisted for at least 7 months. ECHO-6 was also isolated from 50 per cent of the household associates with minor illness and in 16 per cent of those without illness.

The clinical findings (Table 83) were reviewed in 107 patients determined to have ECHO-6 aseptic meningitis by virus isolation. Fever, headache and malaise occurred in almost all cases. Pain, stiffness and muscle spasm especially in the back, the neck and the hamstring muscles were usually present. Approximately one half the cases had abdominal or chest pain. Gastro intestinal complaints, vomiting, anorexia or nausea occurred in approximately 90 per cent of the cases; constipation and diarrhea occurred in less than 15 per cent. Photophobia, sore throat and pharyngeal infection were frequent. Changes in sensorium such as delirium and dis

TABLE 83 CLINICAL FINDINGS IN 107 CASES OF ASEPTIC MENINGITIS DUE TO ECHO VIRUS TYPE 6

General	
Fever	106
Headache	100
Malaise and fatigue	31
Pain	
Muscle pain or spasm	73
Abdominal	43
Chest	17
Gastro intestinal	
Vomiting	81
Nausea or anorexia	11
Constipation	14
Diarrhea	13
Mucous membranes	
Photophobia	24
Sore throat	16
Cough	6
Central nervous system	
Irritability and dizziness	7
Delirium and disorientation	3
Neuromuscular	
Stiff neck and back	99
Weakness	32
Absent superficial reflexes	16
Absent deep tendon reflexes	18

TABLE 82 VIRUS ISOLATIONS FROM 156 CASES OF ASEPTIC MENINGITIS IN ERIE COUNTY NEW YORK IN 1955

VIRUS	STOOL (127)		THROAT (115)		CSF (108)		TOTAL ISOLATIONS (136)	
	No Pos	PER CENT	No Pos	PER CENT	No Pos	PER CENT	No Pos	PER CENT
ECHO 6	78	61%	48	42%	8	8%	92	68%
Polio virus	6	5%	4	3%	0	0	6	4%
Other enteric	5	4%	1	1%	3	3%	8	6%
Total	89	70%	53	46%	11	11%	106	78%

SUMMARY

The presently reported outbreak in western New York State in 1955 offers the following evidence that ECHO-6 is a cause of aseptic meningitis (1) a high recovery rate of ECHO 6 from aseptic meningitis cases (2) the demonstration of a specific neutralizing antibody response (3) *the isolation of ECHO-6 from a* parenteral site the cerebrospinal fluid and (4) the demonstration of an epidemiologic association of ECHO 6 with aseptic meningitis cases and their households in a defined outbreak

The ECHO-6 virus group has been shown to be highly heterogeneous antigenically A mechanism of serologically directed variation of strain pattern has been proposed to account for this observation

BIBLIOGRAPHY

Davis D C and Melnick J L Association of ECHO virus type 6 with aseptic meningitis Proc Soc Exper Biol & Med 92 839 1956

Karzon D T Barron A L Winkelstein W Jr and Cohen S Isolation of ECHO virus type 6 *during outbreak of seasonal aseptic* meningitis J A M A 162 1298 1956

Kibrick S Melendes L and Enders J F Clinical associations of enteric viruses Ann. New York Acad Sc 67 311 1957

Winkelstein W Jr Karzon D T Barron A L and Hayner N S Epidemiologic observations on an outbreak of aseptic meningitis due to ECHO virus type 6 Am J Pub *Health* 47 741 1957

Benign Aseptic Meningitis

DR HERBERT A WENNER

In 19?5 Wallgren described the clinical picture of acute aseptic meningitis Since that time a variety of filtrable viruses have been found in association with febrile illnesses involving the central nervous system Poliomyelitis Coxsackie lymphocytic choriomeningitis and mumps virus and members of leptospiral species have been listed as major causes of illnesses resembling those described by Wallgren Now as Dr Melnick and Dr Karzon have indicated we have the ECHO group added to the list

Our experience with ECHO virus Type 4 began in July 1955 At that time an illness resembling nonparalytic poliomyelitis occurred in Marshall County Iowa Between mid July and August 9 1955 an estimated ?00 patients were seen by practicing physicians in the area

A preliminary survey of the community during the early days of August by the team from the United States Public Health Service indicated that the current illness was widely distributed in the urban population and in regional small towns

The onset of illness was generally abrupt Fever present in all cases was characteristically benign ranging from 99 to 10? F The febrle period lasted 3 to 5 days Headache was a frequent complaint painful extension of the neck was al o a frequent symptom Myalgia involving the lower extremities was encountered in more than half the patients (5/ ‘) The acute phase of illness lasted 4 to 6 days followed by general malaise for several days thereafter

The physical findings consisted of moderate redness of the pharynx stiffness of the neck and the back and only exceptionally muscle weakness Gross evidence of muscular paralysis was not observed The deep tendon and the superficial reflexes were normal Muscular weakness of a transient and minor nature was observed in leg muscles in 2 patients

An increase in the spinal fluid cells was encountered in 16 hospitalized patients The total cell count ranged between 16 and 900 cells per cubic mm the average value being 200 cells were predominantly lymphocytes cerebrospinal fluid sugar and protein values generally within the normal range The hi protein value encountered was 67 mg per

In addition to the patients presenting and symptoms of aseptic meningitis a number of persons were encountered with m illnesses Characteristic features of the m illnesses were low grade fever headache throat and irritability—particularly in chil Evidence of meningeal irritation was no countered in these individuals Unfortuna it was not possible to obtain cerebrospinal f on the individuals with minor illnesses

The epidemic ran its course within a pe of about a month The peak incidence occu between July 20 and August 13 1955 based on reported and surveyed cases indic an average attack rate of 19 per cent In urban area of Marshalltown the highest at rates (?6) were encountered in areas of economic status along with a trend for oc rence of secondary cases in larger sized fam Assuming that the population group chosen survey represented a random sample interp tion of the average rate (16 ‘) for Mars town indicated that as many as 3 ?00 individ may have experienced infection with EC Type 4 virus during the course of the epide

The age specific attack rates for frank minor illnesses (Table 86) were highest in dren (31 ‘) Attack rates for adults v lower ranging between 14 and 19 per c Multiple cases occurred in family associ The larger the size of the family group greater was the likelihood of encountering en aseptic meningitis or minor illness The indicate that the household as a group en experienced infection with virus or was spa In many instances infection was an all-or n phenomenon When secondary cases occur within a family group they usually appea

TABLE 86 COMPARISON OF AGE SPECIFIC ATTACK RATES BY TYPE OF ILLNESS (SURVEY CASES ONLY) MARSHALL COUNTY IOWA SUMMER 1955

Age in Years	Survey Population	Cases with Meningeal Symptoms		Associated Minor Illness		Total	
		Number	Rate Per 100	Number	Rate Per 100	Number	Rate Per 100
0–14	*214*	*36*	*16 8*	*30*	*14 0*	66	30 8
15–20	93	10	10 8	8	8 6	18	19 4
30–44	135	15	11 1	5	3 7	20	14 8
45–59	96	4	4 2	9	9 4	13	13 6
60 & over	65	2	3 1	1	1 5	3	4 6
Total	603	67	10 7	53	8 4	120	19 1

within 5 days after onset of the index case Whether this indicates a relatively short incubation period or whether most of these cases were *co primaries could not be accurately determined*

Cytopathogenic agents were recovered in renal cell cultures from 23 of 57 individuals studied (Table 87) Twenty one agents were detected in 57 fecal specimens Two of 7 individuals studied yielded an agent in throat washing The single spinal fluid tested was negative in tissue culture

Nineteen of the 23 agents were specifically neutralized by ECHO Type 4 antisera None of the agents was neutralized by poliomyelitis or Coxsackie antisera Each of the 4 remaining positive fluids was neutralized by a mixture of ECHO Type 4 and poliomyelitis antisera Three specimens yielded in addition to ECHO Type 4 poliomyelitis virus Type 1 and one yielded polio virus Type 3

Serologic evidence that ECHO virus Type 4 was related to illness was obtained with 35 paired sera from sick individuals and their *family associates* A fourfold or greater rise in serum neutralizing antibody was observed in 14 paired sera Three additional sera contained neutralizing antibody at the time when the first serum was obtained and a rise in titer was not observed The remaining 8 individuals did not have specific antibodies in early or later bleedings

If the data are examined according to history of illness (Table 88) the following apply Ten of 13 individuals with aseptic meningitis had an increase of fourfold or greater in homotypic antibodies Of the 3 remaining individuals 1 had pre-existing antibodies without subsequent change the remaining 2 did not have nor did they develop specific antibodies beyond the 1 4 or 1 8 serum dilution tested

TABLE 87 FREQUENCY OF VIRUS RECOVERY FROM 57 INDIVIDUALS BY CLINICAL STATUS MARSHALLTOWN IOWA EPIDEMIC 1955

Clinical Status	Virus Recovery (ECHO 4)		
	Number Individuals	Number Positive	Per cent Positive
Aseptic Meningitis	31	17	55
Minor Illness	15	4	27
No Illness (Familial Contacts)	11	2	18
Total	57	23*	40

Virus recovered from throat washings of 2 patients and from feces of the other 21 Four had mixed infection 3 with ECHO 4 and Polio 1 1 with ECHO 4 and Polio 3

TABLE 88 NEUTRALIZING-ANTIBODY RESPONSE TO ECHO 4 AMONG 25 INDIVIDUALS BY CLINICAL STATUS MARSHALLTOWN IOWA EPIDEMIC 1955

Clinical Status	Number Individuals	Rise 4 x or Greater	No Significant Change in Titer	No Antibody
Aseptic Meningitis	13	10	1	2
Minor Illness	8	2	1	5
No Illness (Familial Contacts)	4	2	1	1
Total	25	14	3	8

Initial dilutions of 1 4 … 1 8

The occurrence of multiple infections in affected households was observed. Two families were studied and are presented here in respect to serologic conversion. One of these families is recorded in Table 89. The illness in this family extended to involve 5 of the 7 members during a brief period; the onset of illness in 4 members occurred within a 4-day interval. Four children had aseptic meningitis; 1 adult experienced a minor illness. ECHO virus Type 4 was recovered from the feces of 2 children with aseptic meningitis; the virus was not detected in the remaining 2 siblings. Stools were not obtained from the children until 15 to 25 days after onset of illness. A rise in serum neutralizing antibodies occurred in sera obtained from the 3 individuals experiencing illness in the household. The second family represents the occurrence of inapparent infection in the family unit. One member of the family became ill with signs and symptoms of aseptic meningitis. None of the other 6 members developed a recognizable illness. ECHO virus Type 4 was found in the feces of 1 of the adults but not in the 3 teenagers. The virus carrier had specific antibodies without subsequent rise, whereas antibody rises were observed in 3 other individuals in the family.

The epidemic of benign aseptic meningitis occurring in Marshall County, Iowa, during the summer of 1955 was associated closely with ECHO virus Type 4. The data do not provide unequivocal evidence that ECHO virus Type 4 was the virus responsible for illness; the recovery of this virus from patients and the subsequent rises in type-specific antibodies strongly suggest

TABLE 89 OCCURRENCE OF INFECTION IN A HOUSEHOLD—THE OM FAMILY—MARSHALLTOWN IOWA EPIDEMIC 1955

Family Members	Age (Years)	Illness History	Date of Onset	Virus Isolation (Stool)	(Date)	ECHO 4 Serum Neutralizing Antibody Titer
OM Sr	26	Minor Illness	8/6	No Specimen	8/10 10/14	<4 >128
IM	25	No Illness	—	No Specimen	No Specimen	
JM	8	Aseptic Meningitis	7/28	8/12 ECHO 4	8/10 10/14	10 82
OM Jr	6	Aseptic Meningitis	7/26	8/18 Negative	8/10 10/14	16 >128
JKM	5	Aseptic Meningitis	7/24	8/18 Negative	No Specimen	
RM	3	No Illness	—	No Specimen	No Specimen	
MM	2	Aseptic Meningitis	7/24	8/18 ECHO 4 Polio-1	No Specimen	

Titer expressed as reciprocal of final serum dilution

TABLE 93 COXSACKIE VIRUSES NEW TYPES

GROUP	COLLECTION NUMBER	CYTOPATHOGENICITY			OTHER CHARACTERISTICS
		MONKEY KIDNEY CELLS	HELA CELLS	UTERINE PLASMA CLOT	
A	5536	−	+*	−	Isolated in Louisiana † Serologic cross reactivity with serum of strain No 55166 Not identical
A	55161	−	+	+	Isolated in California Induces paralysis in some 10 to 12 Gm mice‡
A	55166	−	+	−	Isolated in New York State on HeLa cells Serologic cross reactivity with serum of strain No 5536 Not identical
A	55222	+	−	−**	Isolated in Italy on monkey kidney cells aseptic meningitis epidemic§ Cross neutralization with serum prepared with ECHO Type 9 representative strain▲
A	5630	−	−	−	Isolated in New York State in suckling mice
B	5592	+	−	−	Representative strain of ECHO 10 (Sabin) △ Grows in eggs

Not cytopath genic w h n fi t te t d on HeLa cells
Other str in of th typ cyt p thogeni on hum n ute ine cells Titer 1/
† Kilbou ne E D and Goldfield M Am J Med 21 1/5 1956
‡ Dalldorf G The neu opathog n ity of Group A C xs ckie es J Exp r Med 106 69 195/
§ Ar hetti I nd F I A Riv Ist roterap ital 31 265 1956
▲ Committee on th ECHO V ru S ience 122 1187 1955
△ Sh w M N w York State Dept of Health D of Labo ato ies and Re e ch Ann l Rep rt 1956

Table 92 lists the 5 viruses in Group B and they are readily handled in tissue-culture testing although there are differences Generally monkey kidney cells are very satisfactory in picking up the B s

Table 93 shows the additional types that have not been assigned type numbers as yet Type 55222 which we first received from Italy and which has been mentioned previously as ECHO 9 is quite similar to A 9 in its behavior in tissue culture Of the others some of them are rather similar to the higher types of A that appeared in Table 91

Our present practice for isolation of infectious agents from the feces of patients makes use of both tissue culture and suckling mice The tissue cultures include trypsinized monkey kidney cells HeLa cells and human uterine tissue in plasma clot We hope soon to include human amnion cell culture as well We have found that the spectrum of cytopathogenicity obtained frequently gives us a clue to the sera for use in our preliminary screening test The suckling mice are 1 day old or less since some strains are not readily isolated even in 2-day-old animals Inoculations are made by 3 routes First passage is also made in 1 day-old mice using both brain and muscle by the same 3 routes—intracerebral subcutaneous in the interscapular area and intraperitoneal

For some strains usually Group B repeated passage is necessary before all mice are uniformly infected and a neutralization test is possible For these passages with Group B strains we use the subcutaneous route and both brain and muscle tissue For Group A the intraperi

[TA]BLE 94 VIRUS STRAINS ISOLATED FROM 308 SEWAGE SPECIMENS INOCULATED INTO NEWBORN MICE AND MONKEY KIDNEY OR HELA CELL CULTURES

Agent	Series A: Mice Only	Series A: HeLa Cells Only (208 Specimens)	Series A: Simultaneously in Mice and HeLa Cells	Series B: Mice Only	Series B: Monkey Kidney Tissue Only (100 Specimens)	Series B: Simultaneously in Mice and Monkey Kidney Tissue
Coxsackie A	63	0	0	41	0	0
Coxsackie B	6	3	1	9	10	10
Poliomyelitis	0	64	0	0	33	0
Orphans	0	3	0	0	2	0
All Agents	69	70	1	50	45	10

toneal route is used with muscle suspension since for many of the newer types the brain suspension contains very little virus.

From our 1956 specimens in only one case was a Coxsackie virus isolated in tissue culture and not in suckling mice. This was a Group A Type 9. However, several Group B Type 3 strains required what was equivalent to blind passage though guided by histologic lesions and might have been missed. One virus, Group B Type 3, was isolated by this method in suckling mice but was not recovered in tissue culture.

Occasionally a Coxsackie virus has proved to be isolated more readily in tissue culture than in mice. It is important to realize that many Coxsackie viruses including a number of the more interesting and possibly more important types may be isolated only in mice. They have not been cytopathogenic for any of the cultured cells so far tested by us. In these days when for practical reasons many laboratories rely largely on tissue culture, it is necessary to remember the present limitations of that method. I recently tabulated the results of a number of large studies in which the viruses were identified and typed by experienced workers. Of a thousand strains approximately one fourth fell in Group B. These presumably would have been cytopathogenic on monkey cells and many but not all on HeLa cells. The other 730 fell in Group A and 95 per cent of them could not be cultivated on tissue culture with our present technics.

This is a serious problem and deserves emphasis. My associate, Dr. Sally Kelly, who is interested in the sanitary aspects of the enteric viruses, has been looking for them in sewage.

Each year's experience has emphasized the differences between the results of the mouse and the tissue-culture tests. In a recent summary she reported the results of testing 308 sewage samples that yielded 245 enteric viruses (Table 94). Of these 119 were isolated only in suckling mice, 115 only in tissue culture and 11 by both methods.

Similar results have been reported from Dr. Rhodes's laboratories where the specimens were tested in mice, tissue culture and monkeys. At first suspended cell cultures of monkeys' testis were used but later the specimens were retested on monolayers of monkey kidney cells. The final results as reported by Dr. Beale and his colleagues illustrate the importance of the technic.

NUMBER OF STRAINS OF COXSACKIE AND POLIO VIRUSES ISOLATED

	In Mice	In Monkey Testis	In Monkey Kidney	In Monkeys
Coxsackie viruses	18	9	13	0
Polio viruses	0	3	3	5

Only half of the Coxsackie viruses could be isolated on monkey testis and only two thirds on monkey kidney cell cultures. The kidney cells were more susceptible to the Group B viruses. The comparative results in monkeys and tissue culture are equally interesting. 2 polio viruses were isolated in monkeys that were

TABLE 93 COXSACKIE VIRUSES NEW TYPES

Group	Collection Number	Cytopathogenicity			Other Characteristics
		Monkey Kidney Cells	HeLa Cells	Uterine Plasma Clot	
A	5536	–	+*	–	Isolated in Louisiana † Serologic cross reactivity with serum of strain No 55166 Not identical
A	55161	–	+	+	Isolated in California Induces paralysis in some 10 to 12 Gm mice‡
A	55166	–	+	–	Isolated in New York State on HeLa cells Serologic cross reactivity with serum of strain No 5536 Not identical
A	55222	+	–	–**	Isolated in Italy on monkey kidney cells aseptic meningitis epidemic§ Cross neutralization with serum prepared with ECHO Type 9 representative strain▲
A	5630	–	–	–	Isolated in New York State in suckling mice
B	5592	+	–	–	Representative strain of ECHO 10 (Sabin) △ Grows in eggs△

* Not cytopathogenic when first tested on HeLa cells
** Other strains of this type cytopathogenic on human uterine cells Titer low
† Kilbourne E D and Goldfield M Am J Med 21 1 5 1956
‡ Dalldorf G The neuropathogenicity of Group A Coxsackie viruses J Exper Med 106 69 1957
§ Archetti I and Felici A Riv Ist sier terap ital 31 265 1956
▲ Committee on the ECHO Viruses Science 12 118 1955
△ Shaw M New York State Dept of Health Div of Laboratories and Research Annual Report 1956

Table 92 lists the 5 viruses in Group B and they are readily handled in tissue-culture testing although there are differences Generally monkey kidney cells are very satisfactory in picking up the Bs

Table 93 shows the additional types that have not been assigned type numbers as yet Type 55222 which we first received from Italy and which has been mentioned previously as ECHO 9 is quite similar to A 9 in its behavior in tissue culture Of the others some of them are rather similar to the higher types of A that appeared in Table 91

Our present practice for isolation of infectious agents from the feces of patients makes use of both tissue culture and suckling mice The tissue cultures include trypsinized monkey kidney cells HeLa cells and human uterine tissue in plasma clot We hope soon to include human amnion cell culture as well We have found that the spectrum of cytopathogenicity obtained frequently gives us a clue to the sera for use in our preliminary screening test The suckling mice are 1 day old or less since some strains are not readily isolated even in 2-day-old animals Inoculations are made by 3 routes First passage is also made in 1 day old mice using both brain and muscle by the same 3 routes—intracerebral subcutaneous in the interscapular area and intraperitoneal

For some strains usually Group B repeated passage is necessary before all mice are uniformly infected and a neutralization test is possible For these passages with Group B strains we use the subcutaneous route and both brain and muscle tissue For Group A the intraperi

TABLE 94 VIRUS STRAINS ISOLATED FROM 308 SEWAGE SPECIMENS INOCULATED INTO NEWBORN MICE AND MONKEY KIDNEY OR HELA CELL CULTURES

	Series A			Series B		
Agent	Mice Only	HeLa Cells Only (208 Specimens)	Simultaneously in Mice and HeLa Cells	Mice Only	Monkey Kidney Tissue Only (100 Specimens)	Simultaneously in Mice and Monkey Kidney Tissue
Coxsackie A	63	0	0	41	0	0
Coxsackie B	6	3	1	9	10	10
Poliomyelitis	0	64	0	0	33	0
Orphans	0	3	0	0	2	0
All Agents	69	70	1	50	45	10

toneal route is used with muscle suspension since for many of the newer types the brain suspension contains very little virus

From our 1956 specimens in only one case was a Coxsackie virus isolated in tissue culture and not in suckling mice This was a Group A Type 9 However several Group B Type 3 strains required what was equivalent to blind passage though guided by histologic lesions and might have been missed One virus Group B Type 3 was isolated by this method in suckling mice but was not recovered in tissue culture

Occasionally a Coxsackie virus has proved to be isolated more readily in tissue culture than in mice It is important to realize that many Coxsackie viruses including a number of the more interesting and possibly more important types may be isolated only in mice They have not been cytopathogenic for any of the cultured cells so far tested by us In these days when for practical reasons many laboratories rely largely on tissue culture it is necessary to remember the present limitations of that method I recently tabulated the results of a number of large studies in which the viruses were identified and typed by experienced workers Of a thousand strains approximately one fourth fell in Group B These presumably would have been cytopathogenic on monkey cells and many but not all on HeLa cells The other 730 fell in Group A and 95 per cent of them could not be cultivated on tissue culture with our present techniques

This is a serious problem and deserves emphasis My associate Dr Sally Kelly who is interested in the sanitary aspects of the enteric viruses has been looking for them in sewage Each year s experience has emphasized the differences between the results of the mouse and the tissue-culture tests In a recent summary she reported the results of testing 308 sewage samples that yielded 245 enteric viruses (Table 94) Of these 119 were isolated only in suckling mice 115 only in tissue culture and 11 by both methods

Similar results have been reported from Dr Rhodes s laboratories where the specimens were tested in mice tissue culture and monkeys At first suspended cell cultures of monkey testis were used but later the specimens were retested on monolayers of monkey kidney cells The final results as reported by Dr Beale and his colleagues illustrate the importance of the technic

NUMBER OF STRAINS OF COXSACKIE AND POLIO VIRUSES ISOLATED

	In Mice	In Monkey Testis	In Monkey Kidney	In Monkeys
Coxsackie viruses	18	9	13	0
Polio viruses	0	3	3	5

Only half of the Coxsackie viruses could be isolated on monkey testis and only two thirds on monkey kidney cell cultures The kidney cells were more susceptible to the Group B viruses The comparative results in monkeys and tissue culture are equally interesting ? polio viruses were isolated in monkeys that were

not isolated in tissue culture One of the misses was shown by the mouse tests to be a mixture of a Group B Coxsackie and a polio virus These two are known to interfere in tissue culture and the failure to isolate the polio virus in tissue culture may have been due to interference The monkey being insusceptible to the Group B virus responded to the polio virus that was suppressed in the kidney cells

The other failure was one in which a Group A virus was isolated in mice from an inoculum that caused poliomyelitis lesions in a monkey The monkey virus was not typed It is conceivable as will be showed later that the lesions in the monkey were due to the Group A virus

Therefore the failure to find a virus must be *interpreted in terms of the technics that were* used this qualification applies to mouse tests as well as to the methods and the cells used in tissue culture work For example Johnsson compared the results of mouse tests in which 2 routes of inoculation were used (see bottom of page)

One may see readily what strikingly different results the two technics got and how both technics were required to find most of the viruses there I have not tabulated our own experience of this kind but it would be similar We would not do it precisely as Dr Johnsson has but the principle is right and we certainly agree that the sample he has is representative of our experience

In his experience the intracerebral route was superior for Group B viruses and the intraperitoneal or subcutaneous for Group A I have the impression that we would prefer the subcutaneous route for Group B viruses if we were reduced to a single test

To summarize current tissue-culture technics are inadequate for the isolation of many Group A viruses They are satisfactory in isolating Group B viruses Each method has its limitations and a thorough examination requires the use of several technics I suspect that the thorough investigation of a few patients may be more instructive than testing many by a single method

Our primary interest in the Coxsackie viruses has always been their pathogenicity for man in the nature of the diseases they cause In the beginning there were some who doubted that these viruses were human pathogens and this seemed to be a reasonable position because in fact there were no diseases with which to associate them If there were no diseases caused by Coxsackie viruses the viruses must be nonpathogenic However by the time we met in 1951 suggestive evidence of pathogenicity had begun to accumulate Dr Curnen discussed the subject in Copenhagen It was he who first suggested *that Coxsackie viruses might cause epidemic pleurodynia or* Bornholm disease In 1951 the evidence was incomplete and Curnen was also uncertain whether the association held for particular types or not

We need not go into that subject in detail An important extension to our knowledge of epidemic pleurodynia has come from the Swedish studies where different clinical patterns were found in the young and in adults—aseptic meningitis in the children and pleurodynia in their parents A second significant extension of our knowledge has come from the opposite end of the globe from South Africa where Dr Gear *and his associates discovered* the nature of Group B infections in the newborn We now know of 3 different manifestations of Group B infections frequently fatal myocarditis in the newborn aseptic meningitis in young children and epidemic pleurodynia in adults

In those years when we were interested in *the Group B viruses we felt* that they were the more important but our own experience in the past 2 years has changed our view a little in suggesting that the Group A viruses may have significance beyond what we once thought

Originally they had been isolated from the

Effect of the Route of Inoculation on the Isolation of Coxsackie Viruses*

Group	No Isolated by All Methods	No Isolated by IC Only	No Isolated Only by SC or IP	No Isolated by Either Method
A	83	5	27	51
B	67	23	12	32

Johnsson T A Virological and Clinical Study of Infections with Coxsackie Viruses Stockholm *Uddevalla* Bohusläningen 1955

feces of 2 boys with paralytic poliomyelitis The more that was learned about them the more they resembled the polio viruses But there was one great discrepancy The lesions in experimental animals in suckling mice were exclusively of the striated muscles I had never seen a human disease that resembled the one in mice and the few efforts we made to find such a disease or lesions were disappointing We knew nothing of course of the lesions in man because we had no fatalities to examine

Now and then we found a clue that suggested that certain of the Group A viruses might be neurotropic A few minor lesions had been seen in the spinal cords of newborn guinea pigs and occasionally an adult mouse inoculated intracerebrally with a Group A strain became paralyzed

This has been noted recently with 3 strains representing 3 different Group A types It encouraged us to attempt to adapt these strains to adult mice One of the 3 the representative strain of Group A Type 14 has now been established in adult mice and while the adaptation is incomplete and does not suggest the emergence of a mutant but rather the behavior of Group B strains that have been adapted to adult mice nevertheless it is possible to cause paralysis in three fourths of the animals The virus causes myositis in infant mice and poliomyelitis in adult mice Animals of intermediate ages may show both lesions so far however only traces of the second have been found The virus is neutralized only by homologous antiserum in both infant and adult mice and is not neutralized by antisera for mouse encephalomyelitis encephalomyocarditis or the 3 polio viruses

This strain also causes lesions in monkeys The disease in monkeys has been a mild one thus far without fatalities or frank paralysis but the changes in the neuraxis are unmistakable There seems to be no reason to doubt that this Coxsackie A 14 strain causes nonparalytic poliomyelitis in cynomolgus monkeys and I suspect that it may do the same in man that the human disease is also a mild form of poliomyelitis This of course remains to be proved but judging from our experience with classical poliomyelitis it would seem to be a reasonable assumption

Russian workers have isolated a virus from paralytic and fatal cases of poliomyelitis which they call Type 4 polio virus Professor Chumakov gave this strain to one of my colleagues and we have had no difficulty confirming his observations The virus causes necrosis of striated muscles in suckling mice It is not pathogenic for adult mice In other words it has the characteristics of a Group A Coxsackie virus In monkeys it causes a disease resembling paralytic poliomyelitis The lesions are similar to those caused by the A 14 virus and by the 3 polio viruses We found Chumakov's virus to be an old acquaintance a well known Group A Coxsackie virus Type 7 We have tested 2 of our own A 7 viruses originally isolated in 1949 but reisolated from the original fecal specimens for these tests and find that both of them cause lesions in monkeys The general practice has been to discount the significance of Coxsackie viruses isolated from cases of paralytic poliomyelitis and some of my colleagues suspect that a polio virus may have been present as well as the A 7 in the Russian patients However in view of the monkey pathogenicity this does seem to be a little arbitrary and we should seriously consider the possibility that the Coxsackie virus was responsible

Something more should be said about the lesions which I have mentioned and described by the term poliomyelitis It may be that they can be distinguished from those in monkeys inoculated with the 3 established types of polio virus Indeed I think this is not unlikely My own observations suggest that there are differences But in their essential characteristics all are similar There is clear-cut evidence of degeneration and necrosis of motor neurones in the spinal cord and the medulla there is reaction characterized by perivascular infiltrations and glia cell proliferation in the stroma It is a polio-myelitis We need not be concerned to call it that if we remember that the lesion of poliomyelitis is not specific of infections caused by the polio viruses

Whatever the final answers to these questions may be there is now evidence that several of the Group A viruses can cause disease in adult mice and monkeys and that the disease resembles poliomyelitis There are other reasons to suspect that we may have underestimated the Group A viruses Kilbourne and Goldfield have carefully studied patients with acute infections of the central nervous system and came to that

conclusion Johnsson examined 23 of his patients by means of electro-encephalographs and found that 9 had abnormal patterns Three of the 9 were A 7 infections This clearly means parenchymal damage Finally I may say that the disease in animals agrees very well with the weakness and the transient paralysis that we frequently have seen in association with Group A infections Interestingly one of Kilbourne s patients from whom an A 7 virus was isolated also had transient paralysis Two more severely ill patients were infected with A 9

It should be emphasized however that the clinical data we have as a whole indicate that Group A infections are relatively benign diseases despite their tendency to mimic poliomyelitis We have seen no fatalities nor do I know of a proved case with permanent paralysis Whatever their potentialities these diseases with the possible exception of the Russian cases so far have been benign They have been avirulent forms of poliomyelitis

One characteristic of the Coxsackie viruses *that has long interested us has been the annual* change in the predominant types I mentioned this in 1951 but since then we have seen more striking examples Indeed the year we met—1951—proved to be remarkable for the prevalence of B 3 strains Outbreaks were identified in many parts of the world In 1948 B 1 had been the epidemic type A world wide prevalence of B 3 such as in 1951 has not recurred so far as we know but the experience in 1955 and 1956 when epidemics of aseptic meningitis due to a particular Group A virus occurred in *most* European countries and Canada is equally striking

It is possible that we may see the emergence of new varieties as in influenza but I suspect that we are witnessing a somewhat different phenomenon When poliomyelitis first appeared in epidemic form not so long ago some considered it to be new despite the evidence that it had occurred in ancient times The newness was the newness of epidemic poliomyelitis Whatever the environmental changes that have been responsible for the increase in poliomyelitis during the recent past is it not likely that they may have changed the relationship between man and these other enteric viruses as well and that they too are now for the first time emerging as epidemic diseases? Dr Gear has predicted tha as standards of living are improved so will the incidence of paralytic poliomyelitis increase There is every reason to believe that the same holds true for the Coxsackie viruses

Unfortunately we have little morbidity data. Windorfer by means of personal inquiry documented the prevalence of epidemic pleurodynia in Germany and learned that it is increasingly common and the outbreaks more numerous and larger Last summer California had the biggest epidemic in the history of that state It should be kept in mind that the geographic distributions of epidemic poliomyelitis and pleurodynia are very similar The earliest large epidemics occurred in Scandinavia Indeed the disease was reportable in Denmark for a number of years It is my impression that we see more of these diseases in New York than we did although one cannot count the cases that might not have been brought to our attention years ago Possibly the newness of the disease noted in Europe during the past 2 years and I am thinking principally of the reports that the rash was unique represents another emergence of an old disease in epidemic form Scattered sporadic cases attract little attention but epidemics always impress us Our reactions to epidemics are conditioned by the adventures of our forebears These are topics that may interest future Congresses

BIBLIOGRAPHY

Archetti I and Felici A L agente etiologico di una forma di meningite asettica rescontrata in forma epidemica Riv Ist sieroterap ital 31 265 1956

Beale A J Duncan D Stackiw W Davis N Dempster G and Rhodes A J Further observations on the laboratory diagnosis of aseptic meningitis caused by Group-B Coxsackie virus Canad J Pub Health 47 179 1956

Beeman E A Huebner R J and Cole R M Studies of Coxsackie viruses laboratory aspects of Group A viruses Am J Hyg 55 83 1952

Boissard G P B Personal communication December 4 1956

Brown C M Liddle D C and Tobin J O H Bornholm disease and Coxsackie virus Lancet 1 445 1952

B H S and Tobin J O H Further out of Bornholm disease associated w h kie virus Lancet 2 767 195?

Cole R M Herpangina and the Coxsackie viruses New England J Med 245 677 1951 (Correspondence)

Contreras G Barnett V H and Melnick J L Identification of Coxsackie viruses by immunological methods and their classification into 16 antigenically distinct types J Immunol 69 395 1952

Curnen E C Immunology epidemiology and clinical aspects of Coxsackie virus infections *in* Poliomyelitis Papers and Discussions Presented at the Second International Poliomyelitis Conference pp 121 134 Philadelphia Lippincott 1952

Dalldorf G Brief review of Coxsackie viruses in 1956 Ann New York Acad Sc 67 09 1957

——— The Coxsackie viruses Bull New York Acad Med 26 329 1950

——— The neuropathogenicity of Group A Coxsackie viruses J Exper Med 106 69 1957

Dalldorf G and Sickles G M An unidentified filtrable agent isolated from the feces of children with paralysis Science 108 61 1948

Dempster G and Mitchell S C The isolation of Coxsackie virus from cases of pleurodynia in Canada Canad J Pub Health 45 345 1954

De Paola D Coes P de Burno-Lobo M Vasconcellos J V and Travassos J Ocorrencia de virus Coxsackie no Rio de Janeiro An microbiol Rio de Janeiro 2 89 19 7 53

Domok I Isolation of Coxsackie virus strains from infections occurring in the years 1952 54 and their identification Acta microbiol 3 95 19 5

Fagee Schaeffer L Een epidemie van Bornholmse ziekte in Amsterdam Noord (Tuindorp-Oostzaan) Nederl tijdschr geneesk 95 2939 1951

Gabinus O Gard S Johnsson T and Poldre A Studies on the etiology of epidemic pleurodynia (Bornholm disease) Arch ges Virusforsch 5 1 1952

Gard S Aseptic meningitis and Coxsackie viruses Journal Lancet 74 299 1954

Gear J H S Distribution of antibodies to poliomyelitis virus in the general population *in* Poliomyelitis Papers and Discussions Presented at the Third International Poliomyelitis Conference pp 137 148 Philadelphia Lippincott 1955

Huebner R J Cole R M Beeman E A Bell J A and Peers J H Herpangina etiologic studies of a specific infectious disease J A M A 145 628 1951

Huebner R J Risser J A Pell J A Beeman E A Beigelman P M and Strong J C Epidemic pleurodynia in Texas a study of 22 cases New England J Med 248 267 1953

Johnsson T Studies on the etiology of Bornholm disease (epidemic pleurodynia) II Epidemiological observations Arch ges Virusforsch 5 401 1954

——— A Virological and Clinical Study of Infections with Coxsackie Virus 30 pp Stockholm Uddevalla Bohuslaningen 19 5

——— A Virological Clinical and Epidemiological Study of Coxsackie Virus Infection 40 pp Stockholm Uddevalla Bohuslaningen 1955

Johnsson T and Lundmark C A histopathological study of Coxsackie virus infections in mice Arch ges Virusforsch 6 262 1955

Kelly S Winsser J and Winkelstein W Jr Poliomyelitis and other enteric viruses in sewage Am J Pub Health 47 72 1957

Kilbourne E D and Goldfield M Coxsackie viruses and virus like diseases of the adult a three year study in a contagious disease hospital Am J Med 21 175 1956

Lazarus A S Johnston E A and Galbraith J E An outbreak of epidemic pleurodynia with special reference to the laboratory diagnosis of Coxsackie virus infections Am J Pub Health 42 20 1952

LeBouvier G L Interference and cell protection by poliomyelitis virus in tissue culture Nature 174 649 1954

Melnick J L Kaplan A S Zabin E Contreras G and Larkum N W An epidemic of paralytic poliomyelitis characterized by dual infections with poliomyelitis and Coxsackie viruses J Exper Med 94 471 1951

Montgomery J Gear J Prinsloo F R Kahn M and Kirsch Z G Myocarditis of the newborn an outbreak in a maternity home in Southern Rhodesia associated with Coxsackie Group-B virus infection South African M J 29 608 1955

Patz I M Measroch V and Gear J Bornholm disease pleurodynia or epidemic myalgia an outbreak in the Transvaal associated with Coxsackie virus infection South African M J 27 397 1953

Rhodes A J Clark E M, Wood W Shimada F T Silverthorne N M Kendry J B J, Duncan D, and Anglin C S Studies on poliomyelitis in Ontario VI Additional laboratory studies on cases of clinical poliomyelitis excreting Coxsackie virus Canad M A J 68 438 1953

Rita G Russi M and Fischer Fantuzzi L Isolamento di virus Coxsackie da focolai epidemici Marchigiani di meningite asettica acuta Riv Ist sieroterap ital 31 266 1956

Shaw M Cultivation of Coxsackie virus in embryonated eggs and in chick tissue cultures Proc Soc Exper Biol & Med 79 718 1952

Shwartzman G and Aronson S M Participation of brown fat in pathogenesis of experimental poliomyelitis of monkeys Proc Soc Exper Biol & Med 86 767 1954

Sickles G M and Dalldorf G Unpublished data

Sickles G M Mutterer M Feorino P and Plager H Recently classified types of Coxsackie virus Group-A behavior in tissue culture Proc Soc Exper Biol & Med 90 529 1955

Swain R H A and Mitchell R C The isolation of a Coxsackie virus from two cases of Bornholm disease Brit M J 1 1354 1953

Taylor R Personal correspondence Sept 16 1954—May 16 1955

Tyrrell D A J and Snell B Recovery of a virus from cases of an epidemic exanthem associated with meningitis Lancet 2 1018 1956

Vasconcellos J V Goldgheil R Schwarz N M Goes P de and Travassos J Ocorrencia de virus Coxsackie no Rio de Janeiro VII Tipos sorologicas das amostras isoladas na mesma cidade An microbiol Rio de Janeiro 3 139 1954 55

Vivell O Zur Epidemiologie der Coxsackie A *Virusinfektionen in* Deutschland Deutsche med Wchnschr 81 405 1956

Warin J F Davies J B M Sanders F K and Vizoso A D Oxford epidemic of Bornholm disease 1951 Brit M J 1 1345 1953

Windorfer A and Schricker H H Untersuchungen uber das Vorkommen der Bornholmer Krankheit in Deutschland Ztschr Hyg 142 141 1955

Epidemics of Aseptic Meningitis Due to Coxsackie B-5 Virus

DR MICHAEL L FURCOLOW

The Coxsackie viruses which have been reviewed so well by Dr Dalldorf have been responsible for many types of illness including epidemics of aseptic meningitis However outbreaks of this disease due to Coxsackie Type B 5 have not been described in the literature I shall give a preliminary report on one major epidemic and several small ones due to Coxsackie P 5 which occurred in the central United States

Figure 137 illustrates the frequency of isolations of Coxsackie B 5 virus using monkey kidney tissue cultures from cases of suspected poliomyelitis during the summer of 1956 The denominator of the fractions shown represents the number of stools tested from each state and the numerator represents the number of isolations of B 5 The dots represent single isolations Note that the Coxsackie B 5 virus isolations were limited essentially to the 4 northern states although in Oklahoma the number of cases studied was small In Arkansas no isolations were made from 104 individuals The greatest number of isolations came from Iowa where Coxsackie in B 5 infection occurred in epidemic proportions in several areas of the state The virus was recovered from 144 individuals 73 of whom were from one county—Cerro Gordo County—where an extensive investigation was carried on The isolations from Nebraska were mainly from the largest city In Missouri and Kansas the isolations were scattered Only 3 strains of polio virus were recovered from Iowa while poliomyelitis virus was recovered frequently from the patients in the other states

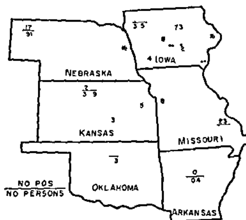


Fig 132 Frequency and distribution of Coxsackie B 5 isolations from stools collected in 6 midwestern states in 1956 The numerator represents the number of individuals with isolations and the denominator the total number of individuals studied

DESCRIPTION OF THE MAJOR EPIDEMIC

The epidemic in Cerro Gordo County Iowa was first noticed in Mason City the county seat Mason City has a population of 33 000 and is located in the central part of the county which contains a population of 46 053 The occurrence of an epidemic illness in this county was first suspected due to reports of increasing incidence of nonparalytic poliomyelitis during the second and third weeks of July

The study in Cerro Gordo County was designed to (1) define the clinical characteristics of the disease and (2) estimate the incidence of the disease and characterize the epidemic The clinical characteristics of the disease were defined by studies of reported cases and their families Any case reported by a physician was considered a reported case These included both hospitalized and nonhospitalized cases

The incidence of the disease was estimated by a random household survey encompassing both the city and the rural areas More than 1 500 persons were interviewed using standard questionnaire forms A survey was conducted on August 16 and a second survey was made 6 weeks later to determine whether cases had continued to occur following the decline of reported cases

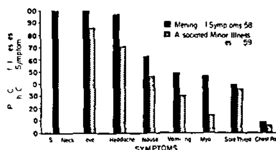


Fig 133 Distribution of symptoms by type of illness (reported cases)

Clinical Picture

The spectrum of illness observed in the epidemic was found to be extremely broad Laboratory evidence of infection was observed in asymptomatic family contacts as well as in those with minor illnesses and aseptic meningitis In general the illness was characterized by fever headache stiff neck nausea and vomiting myalgia and sore throat Figure 133 illustrates the frequency of different symptoms among both the cases with meningeal irritation and those with minor illnesses Stiff neck as well as headache and fever were present in all those cases classified as aseptic meningitis The symptoms among the group classified as associated minor illnesses resembled those seen in the cases with clinical evidence of meningeal irritation

The illness lasted from 2 to 22 days with a median of 9 days The temperature ranged as high as 105 F with a median of 10?° The physical findings were notable for their absence although the patients usually appeared to be acutely ill and nuchal rigidity and injection of the pharynx were commonly seen Although transient muscle weakness was observed no permanent paralysis was noted The clinical laboratory findings were negative except for spinal fluid pleocytosis The spinal fluid cell count ranged from 0 in one case to 2 400 per cu mm averaging 570 Early in the disease polymorphonuclears tended to predominate while during the later stage mononuclears were more numerous Total protein usually was not elevated above normal It is of interest that spinal fluid pleocytosis was found in 7 patients in whom no signs of meningeal irritation could be elicited

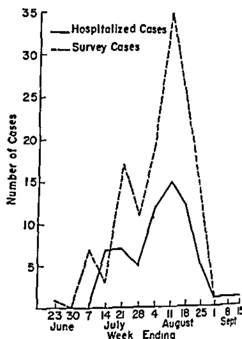


Fig 134 Comparison of hospitalized survey cases by week of onset

EPIDEMIOLOGY

Epidemic Curve

Figure 134 shows the distribution of cases by week of onset for the hospitalized and the survey cases in Cerro Gordo County It is evident that the peak incidence for both groups occurred during the week of August 11 with a majority of the cases appearing during the first 3 weeks of August. Only 3 cases were reported after the last week of August

Attack Rates

Table 95 shows the illness rates observed in a random survey of 1 542 persons in Cerro Gordo County A total of 8 per cent had a history of

Table 95 Incidence and Type of Illness Observed in a Survey Group

Type of Illness	Number	Per Cent
Aseptic meningitis	27	1 7
Minor illness	95	6 2
No illness	1 420	9? 1
Total	1 542	100 0

illness 2 per cent of which could be classified as aseptic meningitis and slightly over 6 per ent as minor illness Based on the incidence rate observed in this survey it was estimated that over 3 000 cases had occurred during the course of the epidemic A resurvey of the same area 6 weeks later revealed no evidence of additional cases after the decline in September

Age Distribution

The incidence of illness in the various age groups is shown in Figure 135 The highest percentage of cases of aseptic meningitis occurred in those 5 to 9 years old On the other hand minor illnesses o curred with equally high frequency among the children in both of the younger age categories In general the age distribution for the two types of illness is similar Approximately two thirds of the cases occurred in the age group under 20 Less than a quarter of the cases occurred among individuals between 20 and 50 and the cases were extremely rare after the age of 50

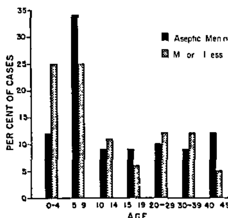


Fig 135 Per cent distribution of all cases by age

ETIOLOGIC STUDIES

Virus Isolations

Spe imens were collected from 152 individuals in Cerro Cordo County These consisted of 133 stools 46 throat washings and 16 spinal fl Sixty seven strains of Coxsackie B 5 were lated from 133 stools 16 from throat wast and 2 from sp nal fluids The frequency of isolations accord ng to type of illness is marized in Table 96 The virus was recov from 63 per cent of the patients with as meningitis from 50 per cent with minor il and from 41 per cent of the family contacts did not have a clinical illness Two of 13 viduals who gave neither history of illness contact with a clinical case also were foun be excreting the virus in their stools

Table 96 Frequency of Coxsackie B 5 Virus Isolations* by Type of Illness

Group	Number of Individuals	Number Positive	Per Cent Positive
Asept c men ng tis	49	31	63
Minor illness	46	23	50
Well family contacts	44	18	41
Well noncontacts	13	2	15
Total	152	74	49

Stool thro t w h gs CSF

The frequency of virus isolation from s among sick and well individuals in those u 20 and over 20 years of age is shown in T 97 It is seen that there was only a slight di ence in the percentage of isolation among individuals in the two age groups Howe among those who had experienced no cli illness the percentage was considerably hi in the age group under 20 than that over years of age

Table 97 Frequency of Coxsackie B 5 Virus Isolation from Stools Among Sick and Well Persons by Age

Age	Sick		Well		Totals	
	Number Tested	Per Cent Positive	Number Tested	Per Cent Positive	Number Tested	Per C Posit
0–19	62	55	22	64	84	57
20 and o er	29	45	23	17	52	33

TABLE 98 NEUTRALIZING ANTIBODY RESPONSE TO COXSACKIE B 5 VIRUS* IN PAIRED SERA BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER OF INDIVIDUALS	ANTIBODY: ABSENT		PRESENT: NO RISE†		PRESENT: RISE‡	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Aseptic meningitis	18	2	11	4	22	12	67
Minor illness	12	2	17	3	25	7	58
Well family contacts	18	5	28	7	39	6	33
Well noncontacts	3	–	—	1	—	2	—
Total	51	9	18	15	29	27	53

* Against 100 TCD_{50}
† Titer of 1 4 or greater with no rise
‡ Fourfold rise or greater

ANTIBODY DETERMINATIONS

Table 98 shows the specific neutralizing antibody response of 51 individuals from whom paired sera were obtained A fourfold or greater rise in titer to Coxsackie B 5 virus between the first and second serum was demonstrated in 53 per cent of the individuals Among 18 cases of aseptic meningitis 12 or 67 per cent showed a rise and 4 or 22 per cent had antibodies but without a rise A rising titer was found in 58 per cent of the patients with minor illness while no change was shown in 25 per cent No antibodies were found in the remaining 17 per cent Among the well family contacts 6 of 18 or 33 per cent showed a rise and 39 per cent had antibodies but with no rise

DISCUSSION AND SUMMARY

During the summer of 1956 several outbreaks of aseptic meningitis were observed in Iowa A large outbreak occurring in Iowa was investigated intensively The results of these studies indicate that the illness was caused by Coxsackie B 5 virus Illness caused by this virus was also prevalent in the surrounding states of Missouri Nebraska and Kansas although apparently not in epidemic proportions

Investigations in Cerro Gordo County indicated that the illness was very widespread involving over 3 000 persons It appears that the disease was most common in persons under 10 years of age suggesting that older persons may have had an immunity as a result of previous contact with the virus

The disease was extremely variable in its clinical manifestations ranging from a mild illness to severe illness with central nervous system involvement Isolation of Coxsackie B 5 virus from a high percentage of the patients with aseptic meningitis as well as from those with minor illness together with the demonstration of specific antibody development suggest that the two illnesses had a common etiology Detection of the virus and specific antibodies in a high proportion of the family contacts further reflects the prevalence of the virus in the community at the time of the epidemic

For two consecutive years 1955 and 1956 epidemics of aseptic meningitis caused by two different viral agents occurred in Iowa These epidemics occurred in two cities Marshalltown and Mason City located 75 miles from each other The 1955 epidemic in Marshalltown was due to ECHO virus Type 4 while the 1956 epidemic in Mason City was caused by Coxsackie B 5 virus These are interesting examples of the periodic appearance of a virus in a given population The periodic occurrence of viruses is further reflected by the studies conducted in our 6 midwestern states during the past 3 years In 1954 no Coxsackie B 5 or ECHO Type 4 viruses were isolated from 250 stools from these states In 1955 ECHO virus Type 4 appeared

in Marshalltown but neither Coxsackie B 5 nor ECHO Type 4 virus was isolated from 300 stools collected from other areas In 1956 Coxsackie B 5 virus appeared in at least 4 of the midwestern states while only 7 strains of ECHO virus Type 4 were found in 650 stools so far processed these strains were isolated from two separate family outbreaks in Kansas

It is evident that these viruses tend to be at times widespread and then disappear entirely from the picture

BIBLIOGRAPHY

Beale A J Duncan D Stackiw W Davis N Dempster G and Rhodes A J Further observations on the laboratory diagnosis of aseptic meningitis caused by Group B Coxsackie virus Canad J Pub Health 47 179 1956

Chin T D Y Beran G W and Wenner H A An epidemic illness associated with a recently recognized enteric virus (ECHO virus Type 4) II Recognition and identification of the etiologic agent Am J Hyg 66 76 1957

Cole R M Bell J A Beeman E A and Huebner R J Studies on Coxsackie viruses observations on epidemiological aspects of Group A viruses Am J Pub Health 41 1342 1951

Curnen E C Shaw E W and Melnick J L Disease resembling nonparalytic poliomyelitis associated with a virus pathogenic for infant mice J A M A 141 894 1949

Dalldorf G The Coxsackie viruses Ann Rev Microbiol 9 277 1955

Dalldorf G and Sickles G M An unidentified agent isolated from the feces of children with paralysis Science 108 61 1948

Huebner R J Cole R M Beeman E A Bell J A and Peers J H Herpangina etiological studies of a specific infectious disease J A M A 145 628 1951

Johnsson T Occurrence of Coxsackie virus infections in an epidemic of poliomyelitis Arch ges Virusforsch 6 216 1955

——— Studies on the etiology of Bornholm disease (epidemic pleurodynia) II Epidemiological observations Arch ges Virusforsch 5 401 1954

Johnsson T and Lindahl J Herpangina a clinical and virological study Arch ges Virusforsch 5 97 1954

Kirby W M M and Evans C A Tissue culture isolation of Coxsackie Group B viruses in aseptic meningitis J A M A 159 743 1955

Lazarus A S Johnston E A and Galbraith J E Outbreak of epidemic pleurodynia special reference to laboratory diagnosis of Coxsackie virus infections Am J Pub Health 42 20 1952

Lehan P H Chick E W Doto I L Chin T D Y Heeren R H and Furcolow M L An epidemic illness associated with a recently recognized enteric virus (ECHO virus Type 4) I Epidemiologic and clinical features Am J Hyg 66 63 1957

Melnick J L Walton M and Myers I L Isolation of a Coxsackie virus during a summer outbreak of acute minor illness Pub Health Rep 68 1178 1953

Stanley N F Dorman D C and Ponsford J A hitherto undescribed group of Coxsackie viruses associated with an outbreak of encephalitis Australian J Exper Biol & M Sc 31 31 1953

Weller T H Enders J F Buckingham M and Finn J J Jr The etiology of epidemic pleurodynia A study of two viruses isolated from a typical outbreak J Immunol 65 337 1950

Encephalomyocarditis Caused by Coxsackie Viruses

DR JAMES H S GEAR

In a graveyard in England on the tombstone of a newborn baby there is the epitaph Since I am so soon done for I wonder what I was begun for Shakespeare did not write that but it is specific nevertheless

Outbreaks of illness occurring in maternity homes or similar institutions are a well known phenomenon Among such infections are diarrhea pneumonitis and myocarditis of the newborn In several recent outbreaks of myocarditis neonatorum the cause of the infection has been identified as a Group B Coxsackie virus

The first outbreak occurred in Johannesburg South Africa in October and November 1952 Ten newborn babies became ill while in or soon after their discharge from the maternity home in which they were born (Table 99) Six of them died and on postmortem examination the cause of death was found to be an acute focal but extensive myocarditis

The illness showed a tendency to be diphasic The first phase was relatively mild and passed unrecognized In one of the fatal cases the infant refused its food and was found to have a temperature from 102° to 104 F in some cases these symptoms were associated with upper respiratory catarrh and in other cases with the passage of loose stools containing mucus After 1 to 3 days the fever subsided This baby appeared to be well until the onset of the second phase 2 to 5 days later when signs of severe illness particularly the signs of myocarditis and circulatory collapse developed

The second outbreak affected 3 babies in another maternity home also in Johannesburg in December 1952 In addition to presenting signs and symptoms of myocarditis these babies also showed clear evidence of encephalitis

The third outbreak occurred in Southern Rhodesia Three babies were affected The evidence which was first obtained that the Johannesburg cases were due to Coxsackie B virus was the pathologic lesions observed in newborn baby mice

Figure 136 is the temperature chart of one of the Southern Rhodesian babies who recovered It is of interest to note that there is a suggestion of a fever somewhat reminiscent of malignant tertian malaria

Figure 137 is the temperature chart of the second Rhodesian baby who recovered

Figure 138 is the temperature chart of the third Rhodesian baby who died A postmortem examination was carried out on this baby and Figures 139 to 142 show the lesions which were observed In the heart (Fig 139) there was a

TABLE 99 OUTBREAK OF MYOCARDITIS NEONATORUM AMONG NEWBORN BABIES IN JOHANNESBURG OCTOBER AND NOVEMBER 1952

CASE NO	BIRTH	ONSET OF ILLNESS	DEATH
1	October 8	October 15	October 20
2	October 14	October 19	Recovered
3	October 17	October 24	November 4
4	October 19	November 5	November 5
5	October 22	October 27	October 31
6	October 22	November 8	November 9
7	October 29	November 7	November 7
8	October 30	November 18	Recovered
9	October 31	November 8	Recovered
10	November 1	November 7	Recovered

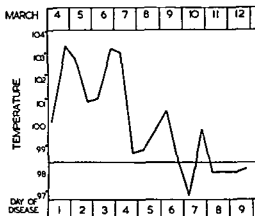


Fig 136 Temperature chart of Baby W born February 25 1954

focal myocarditis shown as a cellular infiltration which was not clearly defined from the surrounding tissues On high power (Fig 140) it was noted that there was some destruction of the muscle associated with an inflammatory cell infiltration mostly of the histiocytic monocytic type The lungs (Fig 141) showed marked edema of the pleura but no inflammation there was marked congestion of the alveolar capillaries but no inflammation although microscopically it looked as though there was intense inflammation The liver showed congestion but no clear

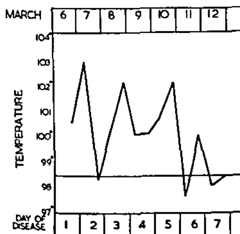


Fig 137 Temperature chart of Baby T born February 26 1954

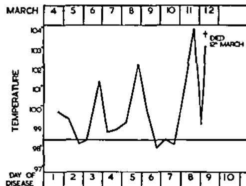


Fig 138 Temperature chart of Baby B born March 2 1954 This case terminated fatally

evidence of focal lesions The kidney did not show any characteristic lesions There was some congestion The suprarenals (Fig 142) showed intense congestion of the medulla which looked as though it was hemorrhagic although the pathologists had some doubt as to whether they were hemorrhages or merely intense congestion In addition there was an inflammatory cell infiltrate in the medulla

Mice inoculated with the feces of 2 of the babies and with the fecal contents of the baby who died developed the characteristic lesions of the fat pad Some mice also showed a focal myocarditis which is characteristic of some strains of myocarditis in baby mice and somewhat reminiscent of the lesions seen in the human babies

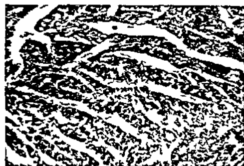


Fig 139 Low power magnification of section of heart muscle showing inflammatory foci (X 75)

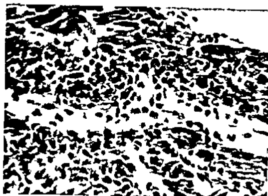


Fig 140 High power magnification of section of heart muscle showing inflammatory degeneration and fragmentation of muscle fibers associated with inflammatory cell infiltrate (×300)

In the Johannesburg outbreaks and in the Southern Rhodesian outbreaks although we obtained evidence of Coxsackie B virus infection we did not prove that the myocarditis was caused by this virus because we did not isolate the virus from the heart muscle In a more recent case which occurred in Johannesburg in 1955 a 9-day-old baby was admitted to the Transvaal Memorial Hospital for Children and died on the day of admission On post mortem examination the baby was found to have again the very characteristic lesions of the heart muscle the focal extensive myocarditis From the heart of this baby a Coxsackie B virus was isolated

From the outbreak in Johannesburg in 1952 a Coxsackie B 3 virus was isolated from the outbreak in Southern Rhodesia a Coxsackie B 4

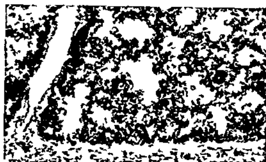


Fig 141 Section of lung showing marked congestion of alveolae and edema of pleura (×75)

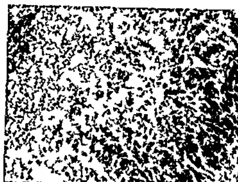


Fig 142 Section of suprarenal showing inflammatory foci in medulla (×75)

virus was isolated from this last case it was a Coxsackie B Type 2 virus which was isolated So it seems that Coxsackie B Types 2 3 and 4 have been incriminated in causing this condition Convincing confirmation of the etiologic role of these viruses has recently come from the workers in Holland Professor van Creveld and his colleagues and Professor Verlinde and his colleagues who are on the panel and will discuss further the role of these viruses

BIBLIOGRAPHY

Bowden K M and French E L Unexpected death in infants and young children *M J Australia* 1 925 1957

Drennan J M Acute isolated myocarditis in newborn infants Arch Dis Childhood 28 288 1953

Javett S N Heymann S Mundel B Pepler W J Lurie H I Gear J and Measroch V Myocarditis in the newborn infant J Pediat 48 1 1956

Keller H Ueber idiopathische Myocarditis im Kindesalter und ihre Differentialdiagnose Helvet paediat acta 1 57 1945

Lind J and Hultquist G T Isolated myocarditis in newborn and young infants Ann Heart J 38 123 1949

MacCallum F O Herpes simplex and vaccinia in early infancy Proceedings VIII International Congress of Paediatrics 1956

Montgomery J Gear J Prinsloo F R Kahn M and Kirsch Z G Myocarditis of the newborn South African M J 29 608 1955

Saphir O Wile S A and Reingold I M Myocarditis in children Am J Dis Child 67 294 1944

van Creveld S and de Jager H Myocarditis in newborns caused by Coxsackie virus Ann paediat 187 100 1956
Verlinde J D van Tongeren H A E and Kret A Myocarditis in newborns due to Group B Coxsackie virus virus studies Ann paediat *187 113 1956*
Weller T H Macauley I C Craig J M and Wirth P Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease Proc Soc Exper Biol & Med 94 4 1957
Williams H O Reilly P H and Williams A Fourteen cases of idiopathic myocarditis *in infants and children Arch Dis Childhood* 28 271 1953

DISCUSSION

DR CONTRERAS I shall comment on one point that has been clearly presented here namely complexity of antigenic structure within ECHO virus types

It has been shown that in ECHO Type 6 besides the prototype D Amori strain there are strains called 6 prime and 6 double prime that show a broader antigenic constitution than the prototype This fact may have a definite immunologic implication

Will you forgive me for presenting a completely theoretical example? Suppose for a moment that an inactive vaccine for this ECHO Type 6 is needed and we have no information about the occurrences of the subtypes Obviously from the facts presented by the previous speakers the immunologic response elicited by Type 6 would give little or no protection against infection by Type 6 When we give an inactive vaccine we give the necessary antigenic mass once and for all and there is no chance that by further multiplication of the virus in the organism that particles of different antigenic structure could come into play as might eventually occur when an active virus vaccine is given I do not say that an active virus poliomyelitis vaccine should be used because first evidence presented here in favor of the inactive virus vaccine is satisfactory secondly no such complex antigenic structure within polio virus types is known to date Nevertheless I shall describe briefly one strain which we have studied in our laboratory

We have been actively engaged in epidemiologic surveys in Chile plus a few samples coming from Argentina and Brazil On the whole we have tested a few hundred stool samples mainly in HeLa cell cultures adapted by us to horse serum some time ago From these samples we have isolated 288 virus strains of which 218 are polio viruses distributed in the following way 80 per cent Type 1 15 per cent Type 2 5 per cent Type 3 Of the remaining 70 strains good evidence indicates that the great majority are ECHO viruses some are Coxsackie viruses which we have not been able to correlate with any particular disease There are also a small number that are adenoviruses and one other strain we have studied thoroughly The possibility that this strain namely Virus 285 is a mixture of polio virus Type 1 with another virus cannot be completely excluded so far and we are reinvestigating this point If it does prove that it cannot be separated into two different viral components it may become an interesting Type 1 strain which could have a similar relation to Prototype 1 as the one *exhibited by* the ECHO 6 to its homologous prototype The importance of such strains in the immunization against poliomyelitis is obvious and *would tend* to emphasize the fact that a diagnostic unit should work side by side with the laboratory preparing vaccine a unit that should keep track of enteric viruses especially among vaccinated persons

Besides the definite pathogenic role played by enteric viruses their complex antigenic structure lends them a particular interest

DR SVEDMYR Dr Melnick in his lecture pointed out that the polio the ECHO and the Coxsackie viruses share many of their properties and indeed in personal communications he has hinted at the possibility of getting a recombination between polio viruses and ECHO viruses Therefore it may be of interest to mention some observations obtained with a complement fixation reaction on human sera that may indicate the existence of an antigenic relationship between polio viruses and ECHO virus Type 6

Most of the ECHO 6 excretors studied by us were found among cases of aseptic meningitis during an outbreak of that syndrome in Stockholm in 1954—a year with an extremely low incidence of poliomyelitis All excretors of Type 6 virus developed neutralizing antibodies and almost all developed complement fixing antibodies against this virus The majority showed a significant rise in titer with at least one of these reactions thus about 50 per cent in the CF titer There was no significant difference whether the *CF antigen was* used live or heat inactivated

However the majority of the Type 6 cases also had comparable titers against heated polio virus antigens of all 3 types and about 50 per cent again showed significant rises in titer al though the rises tended to be somewhat lower than with a homotypic antigen Rises were observed with polio antigens even in cases lacking in their convalescent sera neutralizing antibodies against the 3 types of polio virus Ac tually CF tests with heat inactivated polio antigens gave a much higher proportion of rises in infections with ECHO 6 virus than in polio infections

This crossing phenomenon seems to work in the opposite direction too Thus the majority of the children so far tested who were excreting polio virus Types 1 2 or 3 had CF titers against ECHO 6 antigen whether the latter was used live or heat inactivated The titers were com parable with those obtained with heat inacti vated polio antigens The presence or absence of neutralizing FCHO-6 antibodies among these polio cases made no difference The re sponse was that seen in poliomyelitis cases with the group type heat inactivated polio antigens that is only a few rises in titer were observed

The virus batches used as antigens in these tests were specifically neutralized by prototype antisera and the heat inactivated polio antigens as usual reacted type specifically in a CF test with monkey hyperimmune sera The results seem to suggest that the viruses in question are somewhat related antigenically The fact that the antibody response as measured by the CF reaction seems to be broader in human beings than in test animals corresponds to similar phe nomena within other virus groups like influenza and Coxsackie

I know that Dr Johnsson has been able to onfirm some of these observations and I be lieve also extend them to some other enteric viruses

Dr Hammon In the preceding papers con siderable emphasis has been placed on the role of Coxsackie and ECHO viruses in aseptic menin gitis and in myocarditis Dr Wenner men tioned that he had made serologic tests on sev eral patients to rule out the presence of Western Equine and St Louis viruses also

Where certain of the arthropod borne viral encephalitides are present these in their milder clinical forms present difficult differential diag nostic problems in connection with aseptic meningitis Also they produce aseptic menin gitis In many years of experience in the field in seeing clinical cases of Western Equine and St Louis encephalitis and in attempting to establish a laboratory diagnosis we found that many cases could not be diagnosed in the labo ratory as Western Equine or St Louis encepha litis or mumps or poliomyelitis and we pre sumed that other agents were responsible We know more about those agents today

It has been emphasized that the sharp divid ing lines are rapidly disappearing between polio-myelitis Coxsackie and ECHO viruses Clini cally we know that there are difficulties in making differential diagnosis at least in the milder cases called aseptic meningitis Several speakers have suggested that paralytic cases might be caused by these other viruses I would certainly not be surprised if in the many strains that will be worked with in the future with the many individual persons with different suscep tibilities that severe cases with paralysis would be found due to these other viruses

It has been pointed out that aseptic menin gitis apparently has been caused by ECHO viruses Types 4 5 6 9 and 14 Additional work by Kilbourne and Enders has also indicated that ECHO virus Type 16 can produce an epi demi of aseptic meningitis Coxsackie viruses A 9 B 2 B 3 B 4 and B 5 have also been pointed out as being responsible for these diseases Two speakers have mentioned some difficulties with FCHO Type 13 insofar as an antigenic relation ship with ECHO Type 1 is concerned This virus was isolated in our laboratory in the Phil ippine Islands from Air Force personnel and when we distributed it to other persons appar ently this was a mixture of ECHO Type 1 and ECHO Type 13 Of this we are quite con vinced from recent work that we have done Apparently we have been able to separate this mixture now Whether on original isolation this was a mixture or whether this was contami nated in repeated passage in the laboratory we are not yet sure We have reisolated the virus from the original material It adapts rather slowly and we are not certain However plaques have been picked from the mixture for both Type 1 and Type 13 We are certain that there is a Type 13 that is antigenically di tinct

Dr MacCallum The behavior of infectious diseases in island populations is often of interest and we thought that the information available on ECHO 9 virus infection in Great Britain would be a suitable contribution to this discussion The viruses isolated were identified by cross neutralization tests with the prototype strain and antiserum It is interesting to note that after passage in tissue culture some of the strains isolated in 1955 and 1956 produced myositis and paralysis in newborn mice

In August and September 1954 9 babies were admitted to a hospital in east London each suffering from a similar type of illness which also developed in a baby already in the hospital with a chest infection The most frequent signs of illness were irritability fever vomiting diarrhea and a discrete reddish brown maculopapular rash on the face the trunk and the extensor surfaces of the limbs and the soles of the feet which lasted from 3 to 14 days Red dening of the fauces and enlargement of the superficial lymph nodes were also present Although protein sugar and chlorides in the cerebrospinal fluid were all within normal limits there was an increase in the number of cells varying from 10 to 376 per cu mm of which 95 to 100 per cent were lymphocytes The illness was not serious in any of the babies Stools were received from 6 of the babies acute and convalescent sera from 7 and single convalescent sera from 2 No specimens were received from the first case seen Macrae at the Virus Reference Laboratory Colindale isolated a strain of virus from 5 of the 6 stools by inoculation of tissue cultures of monkey testes and monkey kidney The 5 strains of virus were found to be similar to each other and none were virulent for suckling or adult mice monkeys guinea pigs or rabbit even after passage in tissue culture They were not identified by Macrae as being related to ECHO 9 virus until 1956 Rising titers of neutralizing antibodies to the new viruses were found in sera of 6 babies and high titers in convalescent sera from 2 others No virus was isolated from the stool nor antibodies found in sera from 1 baby There was no clinical evidence that older siblings of the investigated babies had been affected but no laboratory tests were carried out on them General practitioners in the area did not remember having seen any groups of similar cases Two lots of

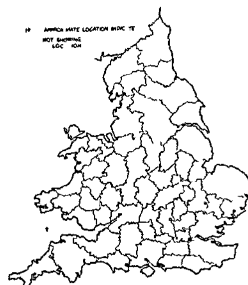


Fig 143 ECHO 9 virus infection in Great Britain

gamma globulin prepared from pools of plasma collected in 1953 from adult donors from London and surrounding areas contained low titers of neutralizing antibody for the virus

Although this laboratory continued to carry out large numbers of isolation tests in monkey kidney cultures on stools from patients with aseptic meningitis there was no indication of the presence of ECHO 9 virus or similar cases of aseptic meningitis with rash until August 1955 It is true of course that an insufficient number of specimens was tested from all parts of the country to say that infection with ECHO virus Type 9 was not occurring From August until December 1955 cases of aseptic meningitis some with a rash were recognized in East Anglia with outbreaks in the Cambridge area ECHO 9 viruses were isolated from the stools the cerebrospinal fluid and the throat washings and there was a rising titer of neutralizing antibody to ECHO 9 viruses in paired sera from patients in the Cambridge Ipswich and Leiston areas

In 1956 investigations of cases of nonparalytic poliomyelitis and aseptic meningitis continued and the first case of ECHO 9 virus infection detected was in a patient from north London in April As far as we know the first definite outbreak commenced in Nottingham in

June In July single cases were detected from County Durham in the north to Surrey in the south as well as many places in between with outbreaks in Lancashire and Leicestershire and possibly other places The disease continued to be prevalent in these areas and new cases appeared in many others so that by the end of November numerous cases of aseptic menin gitis some of which had a rash had been seen in a high proportion of counties of England and Wales and virus was isolated from about 20 of them (Fig 143) I understand that ECHO 9 viruses were also isolated from similar cases in Scotland

At the time of coming to Geneva in 1957 in July there had been no reports of the presence of ECHO 9 virus illness

BIBLIOGRAPHY

Boissard C P B Stokes L J Macrae A D and MacCallum F O Isolation of viruses related to ECHO virus Type 9 from out breaks of aseptic meningitis Lancet 1 500 1957

Crawford M Macrae A D and O Reilly J N An unusual illness in young children associated with an enteric virus Arch Dis Childhood 31 182 1956

Dodd W Lancet 2 947 1956 (Cited in Public Health Editorial)

Eastwood N B An epidemic fever of un known origin in Lowestoft Practitioner (Supp) 177 39 1956

Garnett D C Burlingham A and van Zwanenberg D An outbreak of aseptic meningitis of virus origin in East Suffolk Lancet 1 500 1957

Hughes S B Personal communication 1955 (Quoted by Garnett *et al*)

Kelly F E B Practitioner (Supp) 177 39 1956

Lyle W H Lymphocytic meningo-encephalitis with myalgia and rash a new exanthem? Lancet 2 1042 1956

McLean D M and Melnick J L Association of mouse pathogenic strain of ECHO virus Type 9 with aseptic meningitis Proc Soc Exper Biol & Med 94 656 1957

Rotem C E Meningitis of virus origin Lancet 1 502 1957

Sommerville R Personal communication 1957

Stokes L J and Macrae A D Results of isolation by tissue culture methods during 1956 of poliomyelitis and c viruses Month Bull Ministry H Serv 16 220 1957

Tyrrell D A and Snell B Recovery of a virus from cases of an epidemic exanthem associated with meningitis Lancet 2 10 8 1956

Prof Dr Nihoul A characteristic epidemic of aseptic meningitis which occurred in Belgium in 1956 was proved to have been caused by ECHO virus Type 9 Many of the Belgian ECHO 9 strains are not pathogenic for suckling mice on inoculation of material representin human clinical specimens but acquire such a pathogenicity after one or a few passa es in monkey kidney tissue cultures Based on other similar observations as Dr Melnick sug ested one might consider for purposes of classifi ation the host range of the virus as it naturally exists in human material rather than the host range obtained with strains manipulated in the labora tory However such a criterion in relation to ECHO 9 strains does not seem quite reliable since we have now observed that the few human fecal specimens and one spinal fluid which contained ECHO virus Type 9 were indeed able to provoke histologically detectible myositis in suckling mice on direct inoculation

Similar to the observations of Dr Wenner and Dr Melnick we found antigenic differences between the original ECHO 9 strain from the United States and the European strains of ECHO 9 This fact and the pathogenicity for suckling mice indicate that this group of viruses are heterogeneous but we did not find any anti genic variation between the different European strains of ECHO 9 which so far have been ex amined by ourselves So several Belgian strains isolated at the beginning and the end of the outbreak from different places in Belgium and strains from the Netherlands and Switzerland all were similar when compared in a neutraliza tion test against specific rabbit immune serum

During the study of the Belgian outbreak of aseptic meningitis we observed 14 cases of atypical evolution of the disease Indeed in those cases there were clinical signs of involve ment of the brain and/or of the spinal cord with paralysis In 9 of these cases Mixed infec tion with both polio virus and ECHO 9 virus was —in 4 cases by isolation of both v 5 cases by isolation of one virus anc ation of a rise of antibodies aga In the 5 remaining cases E(ection was proved but the

presence of polio virus could neither be proved nor disproved However analysis of the results suggests that the simultaneous presence of ECHO virus Type 9 and polio virus in the same individual might be more than merely due to chance

The detection of a double infection may become facilitated by the availability in the future of tissue cultures which would be specifically susceptible to infection with ECHO viruses but not to polio viruses We have observed that trypsinized mouse cells are not susceptible to ECHO viruses Type 1 through Type 14 Neither cytopathogenic effect nor multiplication was observed in such tissue as kidney from mice or in kidney muscle or brain from mouse embryo These results are in marked contrast with what was observed in vivo In suckling mice good multiplication of the Belgian ECHO 9 strain was detected in the muscles

Cells in continuous cell culture are not susceptible to ECHO viruses Attempts are now in process to maintain cells in tissue culture originating from kidneys of cynomolgus embryo This culture has been maintained for only 45 days and has undergone 5 subcultures Up to now these cultures have not yet passed through the critical phase In their present status these cells are still as susceptible to ECHO viruses as was the primary culture

Prof Dr Verlinde The 1956 outbreak in the Netherlands of aseptic meningitis due to ECHO 9 virus was extensive and covered practically all regions of the country Family infections were common Virologic and serologic examination revealed that not only a syndrome of meningitis was due to ECHO 9 virus but also that minor illness and a rubelliform rash were common the latter especially in children

This outbreak of meningitis was particularly puzzling for clinicians because of a simultaneous outbreak of poliomyelitis In individual cases it frequently was impossible to differentiate on a purely clinical basis between nonparalytic poliomyelitis and minor illness or meningitis due to infection with ECHO 9 virus

In 1956 the poliomyelitis unit in my laboratory recovered over 1 500 virus strains of these more than 600 were strains of the ECHO group mostly ECHO 9 About 100 were of the Coxsackie group Our group is now collecting the clinical data of the patients examined which is a very difficult and time-consuming job The complete clinical data of 400 patients with a clinical diagnosis of meningeal involvement with or without rash are now available and more will become available before long From these only 10 per cent yielded polio virus and 46 per cent ECHO 9 virus The ECHO strains have been recovered mostly from stools but also from rectal swabs throat swabs and cerebrospinal fluid These materials have been routinely examined by inoculation of trypsinized monkey kidney cell cultures and suckling mice

All strains that took directly in suckling mice proved to be Coxsackie Group A or B viruses All strains that were isolated in monkey kidney culture proved to be poliomyelitis Coxsackie Group B or ECHO viruses The ECHO strains failed to produce any cytopathogenic effect in HeLa cells

The ECHO strains which were neutralized by ECHO Type 9 serum could be divided into two groups according to their pathogenicity for experimental animals Although none of them had been isolated by direct inoculation of suckling mice approximately 70 per cent produced lesions resembling those of Coxsackie A in these animals following inoculation of tissue-culture passage virus

Circumstantial evidence has been obtained that the ECHO Type 9 viruses are responsible for the outbreak of meningitis and sometimes even meningo-encephalomyelitis with transitory paralytic attacks First because of the fact that examination of acute phase and convalescent serum samples from patients as well as from family contacts revealed the development of neutralizing antibodies as evidenced by significant rises in titer Second because of the fact that a Type 9 ECHO virus has been isolated from the intestinal contents as well as from the aseptically removed medulla of a child who died from encephalitis Quantitative virologic examination of the medulla revealed a virus titer of $10^{5.5}$ per gram of tissue

So far 2 strains of ECHO-9 virus one pathogenic for suckling mice the other nonpathogenic for suckling mice have been examined for their pathogenicity for cynomolgus monkeys. Both strains failed to produce clinical signs of illness following intracerebral and intraspinal inoculation of normal monkeys although discrete

lesion were found in the central nervous system In cortisone treated monkeys however the suckling mouse pathogenic strain produced paralysis

Histologic examination of 4 infected monkeys after cortisone treatment showed an intense lymphocytic meningitis covering practically all levels of the central nervous system Moreover in several levels of the spinal cord perivascular infiltrations consisting mainly of mononuclear cells a more or less diffuse or local glial proliferation and a small accumulation of leukocytes were found predominantly in the anterior horns but also around the central canal and even in the posterior horns Although a few nerve cells showed degenerative changes and neuronophagia most of these cells appeared to be normal unlike in poliomyelitis Similar lesions to a lesser extent were found in several parts of the brain for instance the pons the cerebellum the thalamic region and other parts of the brain stem

In cortisone treated monkeys inoculated with a suckling mouse nonpathogenic ECHO 9 virus only a few discrete lesions were found in the pons and other regions of the brain stem

Hence it appears that the viruses of the ECHO group at least those associated with diseases simulating poliomyelitis deserve further attention

However there seems to be little doubt at least during the mixed epidemic of poliomyelitis and viral meningitis associated with ECHO 9 virus in the Netherlands that mixed infections with both viruses in the same individual were not uncommon First from several patients both viruses have been isolated at different occasions second the complement fixation reaction with poliomyelitis antigen might indicate that a considerable number of mixed infections has occurred

Although the complement fixation reaction has been performed in a limited number of the 400 patients from whom complete clinical data are available it is suggested that in approximately half of the patients from whom only ECHO virus has been isolated the poliomyelitis complement fixation reaction with heated Type I antigen was positive This may suggest either a mixed infection or an antigenic relationship between ECHO 9 virus and Type 1 polio virus Nevertheless it seems that ECHO Type 9 virus is an important etiologic agent in aseptic meningitis and in other forms of illness simulating certain forms of poliomyelitis

DR SIGURDSSON Outbreaks of meningitis occurred in Iceland in the period from July to December 1956 The reporting was by no means complete In Reykjavik for instance it is known that the incidence was several times higher than the reports showed In spite of that, in one of the medical districts the incidence was almost 10 per cent

The age incidence was distributed fairly evenly over the various age groups The clinical signs and symptoms were those common for meningitis No deaths occurred One hospital in Reykjavik admitted 29 of the more severe cases Fever lasted for from 2 to 10 days in two thirds of the cases it was from 38 to 39 C and in one third of the cases from 39 to 40 C

No bacteria were found in the spinal fluid of these patients No virus could be recovered from spinal fluid or feces in HeLa cell cultures or in baby or adult mice Twenty two samples of spinal fluid were inoculated into monkey kidney tissue cultures A virus strain for these cells was isolated from 7 of these samples Twenty nine samples of feces were tested in the same way and 7 strains of a similar virus were isolated The 14 isolated strains seemed to be similar as far as can be seen

Five of the new strains were found to be neutralized by convalescent sera from the patients from whom they were isolated but not from acute sera from the same patients The convalescent serum samples from each of these patients also neutralized the virus strains isolated from the other patients This is an indication that the virus strains are immunologically related or identical Two other strains isolated from spinal fluid paralyzed and killed baby mice On the other hand the strains tested were not neutralized by antisera to polio virus Types 1 2 or 3 Attempts to neutralize these strains with antisera to ECHO Types 6 and 9 have not given conclusive results Type 6 antiserum did not neutralize but there was some neutralization by Type 9 These strains have been provisionally grouped as ECHO strains

This evidence suggests that widespread epidemics of meningitis caused by ECHO virus

TABLE 100 DEMONSTRATION OF VIRUSES IN SPECIMENS FROM PATIENTS WITH PARALYTIC POLIOMYELITIS ASEPTIC MENINGITIS AND OTHER DIAGNOSES AT THE HOSPITAL OF INFECTIOUS DISEASES OF ESKILSTUNA IN THE SUMMER AND THE FALL OF 1954

	Age	Total Number Exam	Coxsackie Viruses A	Coxsackie Viruses B	Per Cent A	Per Cent B	Polio Viruses	Per Cent	ECHO Virus Type 6	Per Cent	Non typable Viruses	Per Cent	Total Number Positive Specimens	Per Cent
	0–15	2					2	100						
Paralytic	>15	1					1	100						
poliomyelitis	Total	3					3	100						
	0–15	40	2	5	5	13	1	3	23	58	2	3	33	83
Meningitis	>15	37		3		8			21	57			23	62
	Total	77	2	8	3	10	1	1	44	57	2	3	56	73
	0–15	13							5	38			5	38
Others	>15	17							1	6			1	6
	Total	30							6	20			6	20

O specimen was [illegible] both in suckling mice and human embryonic lung tissue

TABLE 101 COXSACKIE VIRUS ISOLATIONS FROM PATIENTS ADMITTED TO DIFFERENT HOSPITALS IN SWEDEN FROM APRIL TO NOVEMBER 1954 DIAGNOSED AS ASEPTIC MENINGITIS AND OTHERS A FEW COXSACKIE NEGATIVE CASES OF PARALYTIC POLIOMYELITIS ARE EXCLUDED

AGE	DIAGNOSIS		NUMBER EXAM	A 4	A 5	A 7	UNTYPED PROBABLY A STRAINS	PER CENT A STRAINS	B 4	PER CENT B STRAINS
<15	Aseptic meningitis		181		1	16	10	15	11	6
	Others	<5 Cells in C S F	59			1	5	10	3	5
		L P not performed	23		2		2	17	1	4
>15	Aseptic meningitis		129			4	3	5	9	7
	Others	<5 Cells in C S F	111			1		1	6	5
		L P not performed	66	1	1			3	3	5
	Total		569	1	4	22	20	9	33	6

occurred in Iceland in December of 1956 The reported incidence in affected districts was 370 cases per 100 000 In one district it was as high as 10 per cent of the population The reported figures are known to be too low Our observations therefore confirm the findings reported from other countries

DR JOHNSSON In 1954 in a study of an outbreak of aseptic meningitis together with Dr Lycke we isolated ECHO 6 in a high percentage I want to stress the importance of using different ways of isolations For example by using a combination of human embryonic lung trypsinized monkey kidney and suckling mice it was possible to find a probable causal agent in 73 per cent (Table 100 p 237) indicating that no other virus need be considered Besides Coxsackie B-4 we also found Coxsackie A 7 The interest in the latter type has increased since Dr Voroshilova isolated the same type from some cases of paralytic poliomyelitis This type seems to have been rather common in Sweden in 1954 as can be seen in Table 101 where we can see that A 7 was isolated in 9 per cent of children with aseptic meningitis The material examined does not permit a statistical evaluation but it strongly supports a correlation between A 7 and aseptic meningitis especially as there was an increasing titer in 6 of the cases One of the children had rather severe encephalitis symptoms with transient muscular weakness

As in Western Europe ECHO 9 was extremely common in Sweden in the fall of 1956 Thus I personally observed 3 different outbreaks and furthermore Dr Wesslen has isolated 80 ECHO 9 strains from 300 cases of aseptic meningitis in northern Sweden The first case from the first outbreak observed was a boy coming from Holland the biggest epidemic was along the Baltic Sea in northern Sweden where the shipping communications with Holland Pelgium and England are very pronounced The epidemic is still going on and it has now spread all over the country

One of the outbreaks I studied in more detail In this outbreak using both embryonic lung and monkey kidney tissue ECHO 9 was isolated in 83 per cent Of 14 fecal samples tested in both kinds of tissue 86 per cent was positive in lung tissue and 36 per cent in monkey kidney Among 20 lung positive samples Dr Wesslen

TABLE 102 FREQUENCY OF ISOLATIONS OF ECHO VIRUS TYPE 4 BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER EXAMINED	NUMBER POSITIVE IN FECES	COMMENTS
Aseptic meningitis + exanthema	8	4	One case also positive in CSF
Aseptic meningitis	4	4	One case also positive in CSF
Minor illness	1	1	Lumbar puncture not performed
Healthy family contacts	3	3	
Total	16	12	

found all but one to be negative in monkey kidney This figure seems to indicate that our strains were more sensitive for human embryonic lung than for monkey kidney tissue

Primary isolation of ECHO 9 is negative in suckling mice In my material about two thirds were positive after isolation in tissue culture by injection by the subcutaneous route in mice Only one was positive by the intracerebral route However after one blind passage in mice it was possible to adapt some further strains for mice either by passing directly in mice or through tissue culture The above mentioned 13 monkey kidney negative strains isolated in human lung by Dr Wesslen were positive in the former tissue when they were passed from human lung

The European ECHO 9 strains often have been isolated in cases of aseptic meningitis with a rubellalike rash Therefore it was confusing to find the same autumn another outbreak of aseptic meningitis with a similar rash from which ECHO 4 could be isolated As can be seen in Table 102 ECHO 4 was isolated in 75 per cent of the cases and virus was isolated from spinal fluid in 2 cases Family occurrences with a varying clinical picture were common The number of positive isolations was the same in lung as in monkey kidney tissue However it was difficult to demonstrate neutralizing antibodies with the standard technic There were significant titer rises (Table 103) in the complement fixing test using a concentrated antigen Also cross reaction was observed with other ECHO strains and with Coxsackie strains

PROF DR VAN CREVELD In his paper Dr Gear cited the clinical pathologic and virologic studies made by my colleagues and me—to which I add the name of Dekking—in 4 cases of myocarditis of the newborn In all cases a Group B Type 4 Coxsackie virus was isolated from the heart muscle The third case was important it concerned 1 of 2 premature infants who were nursed in the same box shortly after each other Both developed aseptic meningitis with a severe pleocytosis A few days later one of the infants died soon after having shown

TABLE 103 OCCURRENCE OF COMPLEMENT FIXING ANTIBODIES AGAINST ECHO VIRUS TYPE 4 BY TYPE OF ILLNESS

TYPE OF ILLNESS	NUMBER EXAMINED	ANTIBODY — PAIRED SERA: NO RISE NUMBER	ANTIBODY — PAIRED SERA: RISE† NUMBER
Aseptic meningitis + exanthema	5	1	4
Aseptic meningitis	3	1	2
Healthy family contacts	2	1	1
Total	10	3	[illegible]

* T[illegible] of 1 [illegible] o[illegible] gre[illegible]
† Fourfold rise [illegible] greater



Fig 144 Extensive scarring of heart muscle (medium magnification)



Fig 145 Very distinct lymphocytic cellular infiltrates (high magnification)

symptoms of myocarditis The microscopic examination confirmed the diagnosis of myocarditis and meningo-encephalitis foci of inflammation were also seen in the liver Dr Gear also stressed that the mothers of our patients before or after delivery showed an influenzalike disease with high fever

In the period when these cases of myocarditis in the newborn caused by Coxsackie virus were observed in the pediatric clinic of the University of Amsterdam 5 cases with an analogous clinical course were observed in newborns in an obstetric clinic in Amsterdam No virus studies were made in these cases Three of the babies died in 2 an autopsy was made and in both cases a myocarditis and a pericarditis were found The pathologist Professor Deelman investigated the preparations of one case and made the diagnosis of Fiedler s myocarditis a form of myocarditis which more and more is regarded as being of viral origin A sixth newborn fell acutely ill outside the obstetric clinic at home on the eighth day of life and died 1 day later At autopsy of this baby also a Fiedler s myocarditis was found

Epidemiologically all these cases belonged to the same group as our cases During the time of this small epidemic of myocarditis in the newborn caused by Coxsackie virus some cases of myo arditis with lethal course in the newborn were also observed outside Amsterdam

Recently as Dr Ostrowski from Haifa informed me an epidemic of myocarditis in infants in which more than 50 infants were infected and many of them died occurred in Haifa and in some cases a Coxsackie virus was isolated from the heart muscle Dr Falk from the Rambam Government Hospital told me that from January until June of 1957 there were 33 cases and in one case of an acute pericarditis which subsided within a few weeks a Coxsackie B 3 virus was isolated from the pericardial fluid by Dr Bernkopf Therefore the problem is as was also indicated by Dr Gear not at all a local problem In this connection I must also mention the large number of cases of myocarditis in newborns in Munich described by Stoeber in 1952 although no virus was isolated in these cases

I agree with Dr Gear about the necessity of a number of measures to be taken when an epidemic of Coxsackie B virus infection in a population occurs—this especially with regard to the possibility of an outbreak of an epidemic of encephalomyocarditis in the newborns with such a large mortality I stress also the necessity of studying further the possibility of protection of the babies exposed to infection

Dr Gear also briefly cited the reduction of the resistance of adult mice against infection by Coxsackie viruses by the administration of cortisone In this connection the work of Kilbourne and co workers deserves special attention These authors found that extensive and disseminated myocardial necrosis could be produced in adult mice by simultaneous administration of certain strains of Coxsackie virus and cortisone The state of intrinsic resistance of the adult mouse is changed in this way to one of marked susceptibility resulting in death This observation may indeed be of importance for the explanation of

the susceptibility of the myocardium of newborn infants for the Coxsackie virus

The speculation of Kilbourne in this connection on the possible conversion of the benign pleurodynia of adults to a more serious if not fatal disease by the stimulus of endogenous or exogenous corticosteroids surely must be kept in mind and of course it is of very great importance that the adult patient is treated in the clinic for a long time with cortisone

The conclusion seems to be justified that acute myocarditis in older children and adults may be accompanied by far fewer symptoms than in very young infants The prognosis in babies during the acute stage is severe Early treatment with digitalis may promote recovery

When the patient recovers from acute myocarditis restitutio ad integrum takes place but with the exception of diphtheric myocarditis only a few investigations are known However about the occurrence of the contrary namely enlargement of the heart and decompensatio cordis as late consequences of myocarditis several studies have been published

My co worker Dr Hartog and I observed 3 patients with enlargement of the heart and decompensatio cordis in which the postmortem study demonstrated the presence of chronic myocarditis (Figs 144 and 145) These patients died from progressive cardiac failure

In explaining such cases of chronic myocarditis the possibility of recurrent myocarditis caused by different viruses must be considered It is difficult provisionally to give a proof of this possibility

BIBLIOGRAPHY

Blattner P J Comments on current literature acute aseptic myocarditis in the newborn infant J Pediat 50 642 1957

Chaptal J Jean K Loubatières R and Campo Cl A propos de 21 cas d asystolie aigue du nourrisson avec gros coeur etude clinique et anatomique (deux controles histologiques) Arch franç pediat 13 689 1956

Fletcher E and Brennan C F Cardiac complications of Coxsackie virus infection Lancet 1 913 1957

Javett S N Heymann S Mendel B Pepler W J Lurie H I Gear J H S Measroch V and Kirsch Z Myocarditis in the newborn infant a study of an outbreak associated with Coxsackie Group B virus infection in a maternity home in Johannesburg J Pediat 48 1 1956

Kagan H and Bernkopf H Pericarditis caused by Coxsackie virus B Ann paediat 189 1 1957

Kibrick S and Benirschke K Acute aseptic myocarditis and meningoencephalitis in the newborn child infected with Coxsackie virus Group B Type 3 New England J Med 255 883 1956

Kilbourne E D Wilson Ch B and Perrier D The induction of gross myocardial lesions by a Coxsackie (pleurodynia) virus and cortisone J Clin Invest 35 362 1956

Kloosterman C J Annual Report—Vroedvrouwenschool Amsterdam 1956

Kugel M A and Stoloff E G Dilatation and hypertrophy of heart with myocardial degeneration and fibrosis (so-called congenital idiopathic hypertrophy) Am J Dis Child 45 828 1933

Marinesco C Turco I Friedman I Ciurezo V and Draganesco N La myocardite polio myelitique etude anatomo-clinique Semaine hop Paris 33 212 1957

Perrin A Froment R and Lenegre J Insuffisance cardiaque des jeunes sujets par sclerose myocardique dense et diffuse d origine tuberculeuse incertaine ou inconnue le probleme des myocardites subaigues primitives de Josserand et Gallavardin Cardiologia 22 257 and 333 1953

Saphir O Myocarditis general review with analysis of 240 cases Arch Path 32 1000 1941 *ibid* 33 88 1942

Stoeber E Weitere Untersuchungen ueber epidemische Myocarditis (Schwielenherz) des Sauglings Ztschr Kinderh 71 319 and 592 1952

van Creveld S de Groot J W C Hartog H A Ph and Lie Sing Kiem Diagnosis and treatment of acute interstitial myocarditis in infancy Ann paediat 183 193 1954

van Creveld S and de Jager H Myocarditis in newborns caused by Coxsackie virus clinical and pathological data Ann paediat 187 100 1956

Verlinde J D van Tongeren H A E and Kret A Myocarditis in newborns due to Group B Coxsackie virus virus studies Ann paediat 187 113 1956

Studies on Cultured Mammalian Cells

Wednesday Morning, July 10 1957

Chairman

PROF PIERRE LÉPINE
Institut Pasteur
Paris

Speakers

DR HARRY EAGLE
National Institutes of Health
Bethesda Md

DR THEODORE T PUCK
Department of Biophysics
University of Colorado Medical Center
Denver

DR RAYMOND C PARKER
Connaught Medical Research Laboratories
University of Toronto
Toronto

DR ALICE E MOORE
Sloan Kettering Institute for Cancer Research
New York

Discussants

Moderator DR WILTON EARLE
National Cancer Institute
National Institutes of Health
Bethesda Md

DR R S CHANG
Harvard University
School of Public Health
Boston

DR LEWIS L CORIELL
Camden Municipal Hospital
Camden N J

DR RENATO DULBECCO
Division of Biology
California Institute of Technology
Pasadena

DR ANDRÉ LWOFF
Institut Pasteur
Paris

DR WILLIAM F McLIMANS
Wistar Institute for Anatomy and Biology
Philadelphia

DR JOSEPH L MELNICK
College of Medicine
Baylor University
Houston

DR JONAS L SALK
Virus Research Laboratory
University of Pittsburgh
Pittsburgh

DR JEROME T SYVERTON
Department of Bacteriology and Immunology
University of Minnesota Medical School
Minneapolis

Nutritional Requirements for Growth of Cells and Viruses

DR HARRY EAGLE

A number of technical developments in the art of tissue culture have made possible exploration of the nutritional requirements and metabolic activities of a wide variety of animal and human cells. Perhaps the most important single advance has been the provision of cell cultures which can be grown either as monocellular layers adherent to a glass surface and overlaid with a fluid medium or as suspensions of discrete cells. The cells thus are taken out of the tissue and out of a supporting plasma clot and grown in immediate contact with a fluid medium. Their growth rate is increased thereby and their equilibration with the medium facilitated. That medium can be changed at will and the effect of such changes on the rate of cell growth can be determined with precision.

In this manner, determination of the nutritional requirements of a variety of animal and human cells deriving from both normal and malignant tissues has been possible. Since large amounts of cells become available for chemical study, one can explore the metabolic pathways involved in the utilization of these essential metabolites for the biosynthesis of the cellular macromolecules. The data derived from these studies on cellular nutrition and metabolism in turn provide leads to the mechanism of virus synthesis and to the effects of viral infection on cellular metabolism. Thus we come full circle. The technics of the microbiologist have been applied to mammalian cells in pure culture and these studies in turn provide new avenues of exploration for the pathways of viral biosynthesis.

THE AMINO ACID REQUIREMENTS OF CELL CULTURES

If from a medium which contains only the essential requirements for growth one removes a single essential amino acid, the cells develop cytopathogenic changes within a few days and ultimately disintegrate and die. In their early stages these degenerative changes are reversible, i.e., if the missing amino acid is added back to the medium, the cells recover and go on to multiply at a normal rate.

Eight amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine, valine) are known to suffice for nitrogen balance in feeding experiments in man. However, in cell culture at least 13 amino acids have proved to be essential for the survival and the growth of every animal and human cell so far examined.[4] All have been found to require cystine,* tyrosine, arginine, histidine and glutamine* over and above the 8 "essential" amino acids.

Only the L-amino acids are active, and dipeptides have been found to be as active as their component amino acids, indicative of active peptidase activity.[6] Only minor differences have been found between the cell lines derived from various organs, or between cells deriving from normal and malignant tissues, with respect to the amounts of the various amino acids required for optimal growth,[11] and it is of interest that these maximally effective concentrations in vitro closely parallel the serum concentrations of the various amino acids in vivo.

The anomalous requirement for 13 amino acids by serially propagated cell cultures when the human organism requires only 8 is not due to the fact that the cells have altered on prolonged cultivation. Monkey kidney cells in first tissue-culture passage, tested within 48 hours after their removal from the host, before there had been extensive proliferation, required the same 13 amino acids as did the lines which have been serially propagated for years.[9]

In view of the uniform amino acid requirements of the cell strains so far studied, such minor differences as have appeared deserve emphasis.

1. A rat carcinoma cell has been found by McCoy, Maxwell and Neuman[17] to require asparagin in addition to glutamine.

2. A rabbit fibroblast has been found to require serine.[1]

* Qualification with respect to the cystine and glutamine requirements are discussed on page 245.

3 A variant HeLa cell proved capable of hydroxylating phenylalanine to tyrosine[14]

4 The human cell cultures so far studied all require glutamine[13] and glutamic acid does not substitute except at extremely high and nonphysiologic levels Recent observations by Dr Robert DeMars in our laboratory indicate that at those high concentrations there is apparently an adaptive formation of glutamine transferase and synthetase Such adapted cells can then grow for a time in a glutamine free medium containing low concentrations of glutamic acid

5 Unlike any other cell yet tested monkey kidney cells in first tissue-culture passage can use glutamic acid in lieu of glutamine presumably because of the presence of an active glutamine synthetase[9]

6 In confirmation of the findings of Melnick and his co-workers[1] glycine is highly growth stimulatory for monkey kidney epithelium in first tissue-culture passage[9]

7 Recent experiments in this laboratory indicate that cystine should be removed from the list of the essential amino acids It is not formed from methionine in tissue culture but as in the case of *Escherichia coli* and as in the case of a water mold studied by Volkhonsky[1] it can be synthesized by every cell strain so far examined from reduced inorganic sulfur compounds and glucose The pathways involved in that synthesis are under present study

AMINO ACID METABOLISM IN TISSUE CULTURE

The primary function of most of the essential amino acids is for incorporation into cellular protein With only a few exceptions at physiologic levels there is no significant metabolic breakdown or interconversion[14] Glutamine constitutes a special case which deserves emphasis because of its special position in virus synthesis Like any other essential amino acid it is incorporated directly into protein[16] However in addition it is used for the biosynthesis of glutamic acid aspartic acid proline and asparagin and in a limiting medium both the carbon and the amide nitrogen are used in the biosynthesis of the nucleic acids[?]

There are 2 additional aspects of amino acid metabolism of significance in relation to virus synthesis One is the presence in all human cell strains examined of a large cellular pool of amino acids at concentrations approximately 10 times that of the surrounding medium[19] The second is the fact that in contrast to bacteria there is active intracellular turnover of protein i e the cell protein is constantly breaking down and being resynthesized at a rate of approximately 1 per cent per hour[14] This intracellular turnover could be of importance in relation to virus synthesis

VITAMIN REQUIREMENTS OF CELL CULTURES

To date only 8 vitamins have been shown to be essential for cells in culture The first 7 were vitamins of the vitamin B complex (choline folic acid nicotinamide pantothenate pyridoxal thiamine riboflavin) In addition meso-(myo-) inositol known since 1928 to be an essential growth factor for certain yeasts and fungi and long suspected as playing an important role in animal metabolism has proved to be rigorously essential for the survival and the growth of 20 out of 21 cell lines examined[1] Of the 9 isomers of inositol only 1 myo inositol is active in promoting growth

Specific vitamin deficiencies can be produced in cell cultures by withdrawing a single vitamin and these deficiencies can be cured by the restoration of the missing factor The conjugated cofactors (citrovorum factor diphospho- and triphospho-pyridine nucleotide coenzyme A pyridoxal cocarboxylase and flavin mono and dinucleotide) substitute for the corresponding vitamins[7] their occasionally low activity relative to the parent vitamin presumably reflects a difference in cellular permeability

CARBOHYDRATE REQUIREMENTS IN CELL CULTURES

With respect to carbohydrates glucose is essential for cell survival and growth in culture both as a source of energy and as a metabolite for the synthesis of the nutritionally nonessential amino acid (glycine serine and alanine) carbohydrates lipids and nucleic acids However it is not uniquely active Every cell so far studied has proved to be capable of utilizing fructose and mannose in lieu of glucose similarly many can use galactose and ribose and a number of disaccharides have also proved to be active While differences have been observed with respect to the pattern of metabolism with these

various sugar substrates With glucose and mannose there is an active aerobic glycolysis the amount depending on the concentration in the medium However with other substrates and in particular with galactose there is but little fermentative metabolism and the pH of the medium remains relatively stable during the growth of the culture Correspondingly relatively little of these substrates is metabolized in the process of growth[8]

Thus a total of 28 nutritional factors have been shown to be required for the survival and the growth of most mammalian cells in culture 13 amino acids 8 vitamins glucose and 6 ions (Na^+ K^+ Ca^{++} Mg^{++} $H\,PO_4^-$)*[3] In addition a small amount of serum protein must be added to the medium The nature of the essential component(s) supplied by the protein remains to be determined

VIRUS PROPAGATION

What bearing do these results have on propagation of viruses? Of the 28 components essential for the survival and the growth of animal and human cells how many are similarly essential for viral propagation? To what extent can the cell draw on its own components for viral synthesis rather than on the medium? Are the viral protein and nucleic acids synthesized de novo from the amino acids and nucleotides of the cellular pool or are the cellular proteins and nucleic acids partially degraded and reutilized? Only a few of these questions can be answered now although one may reasonably hope that additional information will develop rapidly

The nutritional requirements for viral synthesis have been worked out in detail in one model system the poliomyelitis virus growing in the HeLa cell In this model system the only components of the medium required for virol synthesis other than salts were glucose and glutamine[10] In a medium containing all the essential amino acids other than glutamine all the essential vitamins salts and serum protein supplemented with the 6 nonessential amino acids free ammonia purines and pyrimidines but lacking glucose and glutamine the amount of virus elaborated by HeLa cells was reduced to one ten thousandth of the amount formed in a complete medium Conversely in a medium containing only glucose glutamine and salts and lacking all of the other components required for cell survival essentially normal amounts of virus were formed

In this model system the cell clearly forms virus at the expense of its own cell substance The virus proteins are formed in large part either from a pre-existing amino acid pool or by the breakdown of cell protein similarly the virus nucleic acids are formed either from the nucleotide pool or by the breakdown of the cell nucleic acids There is no present information as to whether the role of glucose and glutamine in the propagation of poliomyelitis virus by the HeLa cell consists in the maintenance of the metabolic processes of the cell or whether they are used directly for virus synthesis

Although glutamic acid substituted for glutamine in the elaboration of virus the concentrations necessary for maximum virus output were the same as those necessary for the growth of the cell this despite the fact that the experiments are carried out in a nongrowth medium comprising only glucose glutamine and salts[1] Further more with monkey kidney cultures which can make glutamine from glutamic acid and significant amounts of glutamic acid from glucose or from other amino acids neither glutamine nor glutamic acid was required for the maximum elaboration of poliomyelitis virus[1]

Also with glucose and fructose the concentrations necessary for the maximum propagation of virus were the same as those necessary for the optimum growth of the cell even in a nongrowth medium[1] The virus yield with other sugars is under continuing study

This similarity in the concentrations required for cell growth and for virus propagation so far is limited to glucose and glutamine It does not extend to any of the other amino acids nor does it extend to the vitamins Dr James Darnell and I have recently observed that the depletion of the cellular reserves of nicotinamide thiamine and riboflavin to the point of cessation of growth and extensive degenerative change has had no important effect on the capacity of the HeLa cell to form poliomyelitis virus However pyridoxal deficiency resulted in

* Bicarbonate i n s not nece ary a *n vitro* *l* fa tor Needle s to say th s doe not excl d its ntial r l metaboli m I the ab ence of add d b bi nat t b comes a ailable to the cell by th o dat on of gluco Th role of tra e elements in th tr tion of a m l ell rema ns to be eluci lated

a marked reduction in virus output and the virus forming capacity of the cell could then be restored by the addition of a full complement of amino acids

The quantitative and perhaps qualitative differences in the requirements for cell growth and for virus propagation are illustrated further in the effects of various inhibitors Cyanide and dinitrophenol at concentrations which caused the early death of the HeLa cell had no immediate effect on its capacity to form polio virus

Clearly animal cell cultures provide an elegant and flexible tool not only for the study of cell metabolism but also for the study of the complex process of viral propagation In comparison with the large body of information with respect to bacteriophage what has been learned to date is only the beginning of the beginning but we may look forward confidently to a period of rapid progress in this fascinating and important area

REFERENCES

1 Darnell J F and Eagle H In preparation
2 DeMars R Biochim et biophys acta 27 435 1958
3 Eagle H Arch Biochem & Biophys 61 356 1956
4 ——— J Biol Chem 214 839 1955 J Exper Med 102 37 1955
5 ——— J Exper Med 102 595 1955
6 ——— Proc Soc Exper Biol & Med 89 96 1955
7 ——— Proc Soc Exper Biol & Med 91 358 1956
8 Eagle H Barban S and Levy M In preparation
9 Eagle H Freeman A E and Levy M J Exper Med 107 643 1958
10 Eagle H and Habel K J Exper Med 104 271 1956
11 Eagle H Oyama V I and Levy M Arch Biochem & Biophys 67 432 1957
12 Eagle H Oyama V I Levy M and Freeman A E Science 123 845 1956 J Biol Chem 226 191 1957
13 Eagle H Oyama V I Levy M Horton C L and Fleischman R J Biol Chem 218 607 1956
14 Eagle H Piez K A and Fleischman R J Biol Chem 228 847 195
15 Haff R F and Swim H E Fed Proc 15 591 1956
16 Levintow L Eagle H and Piez K A J Biol Chem 227 929 1957
17 McCoy T A Maxwell M and Neuman R E Cancer Res 16 979 1956
18 Piez K A and Eagle H J Biol Chem 231 533 1958
19 Rappaport C and Melnick J L Fed Proc 16 429 1957
20 Salzman N P Eagle H and Sebring E J Biol Chem 230 1001 1958
21 Volkhonsky M Compt rend Acad d sc 197 712 1933 Ann Inst Pasteur 50 703 1933

Clones from Single Mammalian Cells

DR THEODORE T PUCK

Mammalian biology during the last half century has dealt to a large extent with structure and functions of tissues and organs While it has been recognized for approximately three quarters of a century that the ultimate unit of these structures is the cell most of the methods for study of mammalian phenomena have dealt with many-celled populations Detailed morphologic physiologic and biochemical information has accumulated elucidating specific functions of many tissues and cell aggregates Yet in almost no case is it clear to what extent any given cell is autonomous and to what extent its behavior is governed by its status as a subunit of a particular organ itself tightly integrated into the over all economy of the body Thus the degree to which individual cells of specialized tissues have retained the capacity for unlimited growth the extent to which different cell specializations represent changes of genome or of nongenetic apparatus the potentialities for further specialization or for reversal of differentiation possessed by a particular cell type the nature of stable self reproducing and even symbiotic association of cells and viruses which may be possible the possibilities for genetic modification by processes like random mutation directed transformation transduction and sexual exchange of nuclear or cytoplasmic genetic determinants and the extent to which processes like cancer formation and ageing are manifestations of genetic or physiologic processes localized within individual cells have remained largely unknown

The last 4 decades have witnessed tremendous advances in understanding of genetic and physiologic processes in a wide variety of cells of independent micro-organisms Much of this progress was due to the availability of a simple technic whereby every single cell plated in a semisolid nutrient medium multiplies in isolation to form a macroscopic colony In our laboratory a program was initiated to develop a similar methodology for mammalian cells in hope that the concepts and the operations of quantitative microbiology and microbiological genetics then could be applied to the individual cells of the mammalian body

A plating technic for mammalian cells completely analogous to that used for *Escherichia coli* or *neurospora* was first achieved with the aid of a feeder layer of x irradiated cells Our experiments were guided by the fact that early developments in tissue culture had resulted in routine methods for growth of large inocula of mammalian cells In more quantitative studies, Earle and his co-workers demonstrated specifically the need for mutual co-operation between cells in a massive inoculum These investigators showed that isolated cells did not multiply in a large volume of the nutrient media then employed However single cells when sealed in a capillary tube which conserves diffusible products produced self sustaining colonies Reasoning from these considerations we devised a method whereby single HeLa cells were added to a petri dish containing nutrient medium plus 100 000 additional cells which had been x irradiated so as permanently to block their own multiplication These irradiated cells formed a feeder layer for the viable cell inoculum This technic permitted every one of the cells of the viable inoculum to multiply and form a macroscopic colony as shown in Figure 146 The plating efficiency achieved by this means closely approximates 100 per cent and the reliability and the precision of the results compare favorably with the most quantitative technics available for micro-organisms like *Escherichia coli* or *T2 bacteriophage*

Studies then were initiated to elucidate what function is performed by the irradiated "feeder cell layer in the hope that it could be fulfilled in other ways and the need for the feeder cells thus eliminated It was found that the feeder cells can function in 3 ways (a) they can compensate for specific molecular and nutritional deficiencies in the medium (b) they can neu



Fig 146 Demonstration of how a feeder cell system operates in promoting growth of colonies from single human cells (*Top*) Plate with x irradiated feeder cells alone (actual size) (*Bottom*) Identical plate as above but to which were added 100 unirradiated cells The colony count obtained was 9

tralize toxic agents like antibodies that may be present in the medium which always contains a mammalian serum component and (c) the feeder layer may permit clonal growth of cells which otherwise fail to multiply because of trauma suffered in the course of dispersal into the single state by methods involving excessive mechanical or chemical trauma When all of these functions are fulfilled by specific adjustments of the nutrient medium and by a sufficiently gentle dispersion procedure the need for feeder cells is eliminated and excellent growth with 100 per cent plating efficiency is obtained

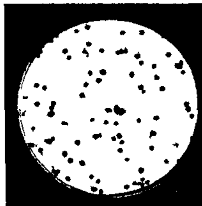


Fig 147 Growth of colonies from single cells under conditions where the need for feeders has been eliminated These are cells from a single clonal strain (S-3) of the HeLa cell originally from a human carcinoma of the cervix

from single cells plated directly into petri dishes containing only the appropriate nutritional medium A typical example is shown in Figure 147 In our experience so far newly established cell strains frequently require feeder systems at first for high plating efficiencies of the single cells to be achieved After cultivation in the laboratory which always includes progressive adjustment of the composition of the nutritional medium good plating efficiency is almost always achieved without the need for feeder cells Elimination of the need for feeder cells probably represents a combination of cell adaptation to growth in vitro as well as better matching of the cell's nutritional needs by the adjusted medium It is important to note that with the new nutrient solutions which we have developed it is often possible to obtain clonal growth of single cells isolated directly from tissues of human subjects

APPLICATION OF SINGLE-CELL PLATING TO VARIOUS HUMAN SOMATIC CELLS

These procedures have been applied successfully to human cells originating from a large variety of organs Excellent plating efficiencies have been achieved with cells representing any of the following conditions of origin young or adult individuals normal or cancerous organs

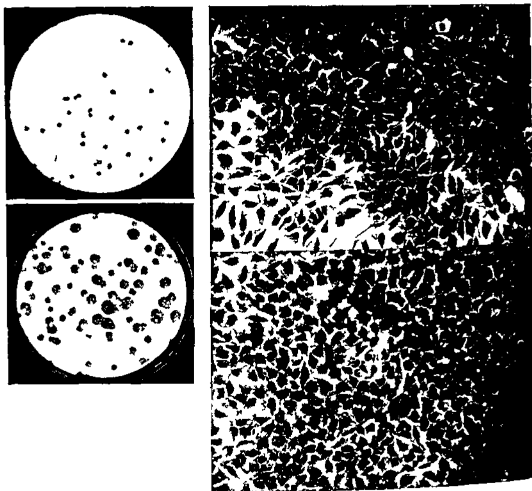


FIG 148 Growth of colonies with epithelial morphology from single cells obtained from a variety of noncancerous human organs (*Top left*) Liver cells (normal size) (*Top right*) Photomicrograph of a single liver cell colony (×170) (*Bottom left*) Conjunctival cells (normal size) (*Bottom right*) Photomicrograph of a single conjunctival cell colony (×200)

newly isolated cells or strains which have been long established in tissue culture morphology resembling that of either epithelial or fibroblast forms and either normal diploid or polyploid chromosomal condition The plating procedures here described have proved to be successful with cells from every human organ which has been tested including liver spleen skin kidney conjunctiva cervix appendix amnion and bone marrow Figures 148 and 149 present the appearance of colonies arising from the plating of single cells of various human organs by means of these technics Clonal cell lines may be established readily by picking any well isolated colony exactly as is done with single colonies of bacteria or single plaques or bacteriophage

These technics permit 3 new types of operations with mammalian cells It makes possible identification of the particular cells in a given population which are capable of sustained multiplication under the defined conditions Since each single cell can multiply in isolation this method also permits ready isolation of rare mutations whose presence would be obscured in op-

FIG 149 Typical colonies with fibroblastlike morphology from normal human organs (*Left*) Colonies from human spleen cells (actual size) (*Right*) Enlargement of colony arising from single cell of human spleen (×75) Cells are spindle shaped and elongated

erations involving large populations and whose divergent behavior then can be used as markers to illuminate genetic processes in mammalian somatic cells Finally it provides a more quantitative and reliable means than was previously available for measuring cell multiplication and the influence thereon of various agents

ANALYTIC STUDIES OF CELL GROWTH

The use of this technic to permit more quantitative study of cellular reproduction is illustrated by the growth curve of Figure 150 which is obtained readily by daily counts of the number of cells in about 20 developing colonies of a plate originally seeded with single cells The initial lag period during which the cells presumably adapt to the medium as well as the subsequent *linear logarithmic increase in numbers* are complete counterparts of the growth curve of *E coli* The construction of such a curve for cells growing under different conditions of nutritional stress or action of physical or chemical agents permits localization of different kinds of actions on the growth cycle Thus some agents reduce the fraction of cells in the population which can multiply others merely increase the lag period Still others may change the slope of the logarithmic part of the curve and finally others may cause the curve to become horizontal after a given amount of growth has been accomplished Various combinations of these actions also occur Studies of the dynamics of mammalian cell growth and the effect thereon of substances with differential action on different cells should profit materially from the use of this technic

In this connection every human cell we have studied so far appears to achieve the same limiting multiplication rate as its growth conditions are steadily improved This maximal rate for sustained reproduction corresponds to a

FIG 150 Typical growth curve of single HeLa cell in complete nutrient medium

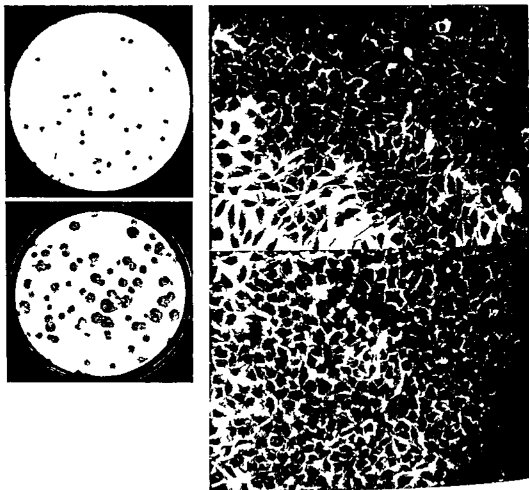


FIG 148 Growth of colonies with epithelial morphology from single cells obtained from a variety of noncancerous human organs (*Top left*) Liver cells (normal size) (*Top right*) Photomicrograph of a single liver cell colony (×170) (*Bottom left*) Conjunctival cells (normal size) (*Bottom right*) Photomicrograph of a single conjunctival cell colony (×200)

newly isolated cells or strains which have been long established in tissue culture morphology resembling that of either epithelial or fibroblast forms and either normal diploid or polyploid chromosomal condition The plating procedures here described have proved to be successful with cells from every human organ which has been tested including liver spleen skin kidney conjunctiva cervix appendix amnion and bone marrow Figures 148 and 149 present the appearance of colonies arising from the plating of single cells of various human organs by means of these technics Clonal cell lines may be established readily by picking any well isolated colony exactly as is done with single colonies of bacteria or single plaques or bacteriophage

These technics permit 3 new types of operations with mammalian cells It makes possible identification of the particular cells in a given population which are capable of sustained multiplication under the defined conditions Since each single cell can multiply in isolation this method also permits ready isolation of rare mutations whose presence would be obscured in op-





FIG. 149. Typical colonies with fibroblastlike morphology from normal human organs. (*Left*) Colonies from human spleen cells (actual size). (*Right*) Enlargement of colony arising from single cell of human spleen (×75). Cells are spindle shaped and elongated.

erations involving large populations and whose divergent behavior then can be used as markers to illuminate genetic processes in mammalian somatic cells. Finally, it provides a more quantitative and reliable means than was previously available for measuring cell multiplication and the influence thereon of various agents.

ANALYTIC STUDIES OF CELL GROWTH

The use of this technic to permit more quantitative study of cellular reproduction is illustrated by the growth curve of Figure 150, which is obtained readily by daily counts of the number of cells in about 20 developing colonies of a plate originally seeded with single cells. The initial lag period during which the cells presumably adapt to the medium, as well as the subsequent linear logarithmic increase in numbers, are complete counterparts of the growth curve of *E. coli*. The construction of such a curve for cells growing under different conditions of nutritional stress or action of physical or chemical agents permits localization of different kinds of actions on the growth cycle. Thus some agents reduce the fraction of cells in the population which can multiply; others merely increase the lag period. Still others may change the slope of the logarithmic part of the curve, and finally others may cause the curve to become horizontal after a given amount of growth has been accomplished. Various combinations of these actions also occur. Studies of the dynamics of mammalian cell growth and the effect thereon of substances with differential action on different cells should profit materially from the use of this technic.

In this connection, every human cell we have studied so far appears to achieve the same limiting multiplication rate as its growth conditions are steadily improved. This maximal rate for sustained reproduction corresponds to a

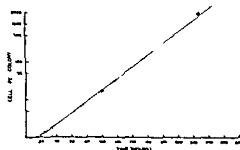


FIG. 150. Typical growth curve of single HeLa cell in complete nutrient medium.

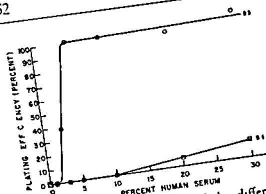


Fig 151 Demonstration of the difference in growth response to human serum of two different mutants which were isolated from the population of the HeLa cell strain obtained from a human cervical carcinoma Use of a basal synthetic medium plus 5 per cent serum gives 100 per cent plating efficiency of the single cells of S-3 and zero for S-1 cells Yet massive inocula of either cell will proliferate even in the low serum medium

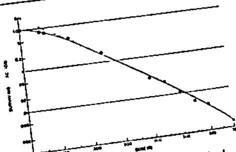


Fig 152 X ray survival curve of a human somatic cell This particular curve is obtained with the HeLa cell as well as with all of the noncancerous human cells so far tested

generation time of 18 hours All but about 40 minutes of this period represents the intermitotic phase

STUDIES ON CELL NUTRITION

Many different investigations attempting to define the nutritional needs of mammalian cells have been described and on the basis of these studies certain key molecular requirements for sustained growth have come to be recognized as common to a variety of mammalian cells cultured in vitro However usually these investigations utilized growth of massive cell inocula The growth requirements obtained by such methods often are uniform for cells obtained from widely different tissues and even for cells which have different morphologies Thus Eagle in his most illuminating studies found as you have just heard that a specific nutritional medium is adequate for support of many kinds of cells Such results appear to be disappointing to the hope that mammalian cells cultivated in vitro might display specific biochemical properties reflecting different functions in the body

When the single-cell plating procedure is employed to study nutritional requirements of a variety of human cells new nutritional requirements often emerge With this technic a variety of differentiated patterns of nutritional requirements has been found Reproducible differences in molecular growth requirements have been demonstrated between morphologically identical cells arising from the same human tissue specimen and from morphologically similar and different cells obtained from different tissues of the same individual Thus for example 2 different clonal cell lines with clearly demonstrable nutritional differences have been found naturally occurring in the population constituting the HeLa cell strain one of the most widely used lines of tissue culture The data of Figure 151 demonstrate that a basal synthetic medium, supplemented with 5 per cent human serum can produce 100 per cent plating efficiency of one strain and no growth whatever of the other

The reason behind the differences in molecular requirements for growth exhibited by single cells on the one hand and massive inocula on the other is of enormous biologic interest Earle first suggested that the decreased nutritional requirements of massive inocula is a reflection of an inability of the biosynthetic apparatus of mammalian cells to keep pace with the loss of diffusible metabolites It would follow from these considerations that differences in nutritional requirements for growth exhibited by single cells from different tissues but not by large cell aggregates is a reflection either of differences in membrane permeability or in the rates of biosynthesis attainable by the various cell types Studies of the differences in the metabolic pattern of single cells as compared with cell populations of varying compositions promises to afford an approach to systematic investigation of different kinds of cell-cell interaction



FIG. 1 Demonstration of giant production from human cells by x-irradiation. (Top) Plate seeded with single cells and then irradiated with [illegible] r. Only two small colonies developed. The remaining cells became giants, each of which is visible to the eye (arrow). (Bottom) Photomicrograph of a normal colony grown among the radiation-damaged cells which are becoming giants. ([illegible])

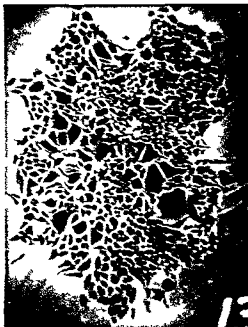


FIG. 1[illegible] Typical colony from the [illegible] of S[illegible] produced as a result of x-irradiation. Each single cell, when plated in nutrient medium, produces a colony studded with monster forms as shown.

in the mammalian body tissues. Under special conditions, such cells may exhibit nutritional biochemical requirements for growth which have not been found heretofore in bacteria. An example of such action is the need exhibited by [illegible] cells of the HeLa S[illegible] clone for [illegible]. The quantitativeness of the single-cell plating technique permits [illegible] of [illegible] for a [illegible] with human cells in a manner [illegible] to that used with the [illegible] in bacteria.

GENETICS AND THE ACTION OF IONIZING RADIATION

This method has been used to measure the action of ionizing radiations on mammalian cells and has permitted determination of their x-ray survival curve, as shown in Figure [illegible]. These experiments have yielded values of the mean lethal dose of radiation for various human cell strains between [illegible] to [illegible] r, a value much lower than that which had been presumed to represent the mean radiation lethal dose for somatic cells. Curves obtained for cells from different organs, including both normal and neoplastic tissues, are very similar. An abundant [illegible]

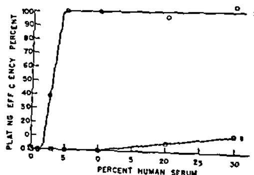


Fig 151 Demonstration of the difference in growth response to human serum of two different mutants which were isolated from the population of the HeLa cell strain obtained from a human cervical carcinoma Use of a basal synthetic medium plus 5 per cent serum gives 100 per cent plating efficiency of the single cells of S-3 and zero for S-1 cells Yet mass inocula of either cell will proliferate even in the low serum medium

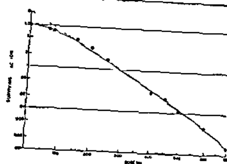


Fig 152 X ray survival curve of a human somatic cell This particular curve is obtained with the HeLa cell as well as with all of the noncancerous human cell so far tested

generation time of 18 hours All but about 40 minutes of this period represents the intermitotic phase

STUDIES ON CELL NUTRITION

Many different investigations attempting to define the nutritional needs of mammalian cells have been described and on the basis of these studies certain key molecular requirements for sustained growth have come to be recognized as common to a variety of mammalian cells cultured in vitro However usually these investigations utilized growth of mass cell inocula The growth requirements obtained by such methods often are uniform for cells obtained from widely different tissues and even for cells which have different morphologies Thus Eagle in his most illuminating studies found as you have just heard that a specific nutritional medium is adequate for support of many kinds of cells Such results appear to be disappointing to the hope that mammalian cells cultivated in vitro might display specific biochemical properties reflecting different functions in the body

When the single-cell plating procedure is employed to study nutritional requirements of a variety of human cells new nutritional requirements often emerge With this technic a variety of different added patterns of nutritional requirements has been found Reproducible differences in molecular growth requirements have been demonstrated between morphologically identical cells arising from the same human tissue specimen and from morphologically similar and different cells obtained from different tissues of the same individual Thus for example 2 different clonal cell lines with clearly demonstrable nutritional differences have been found naturally occurring in the population constituting the HeLa cell strain one of the most widely used lines of tissue culture The data of Figure 151 demonstrate that a basal synthetic medium supplemented with 5 per cent human serum, can produce 100 per cent plating efficiency of one strain and no growth whatever of the other

The reason behind the differences in molecular requirements for growth exhibited by single cells on the one hand and mass inocula on the other is of enormous biologic interest Eagle first suggested that the decreased nutritional requirements of mass inocula is a reflection of an inability of the biosynthetic apparatus of mammalian cells to keep pace with the loss of diffusible metabolites It would follow from these considerations that differences in nutritional requirements for growth exhibited by single cells from different tissues but not by large cell aggregates is a reflection either of differences in membrane permeability or in the rates of biosynthesis attainable by the various cell types Studies of the differences in the metabolic pattern of single cells as compared with cell populations of varying compositions promises to afford an approach to systematic investigation of different kinds of cell-cell interaction

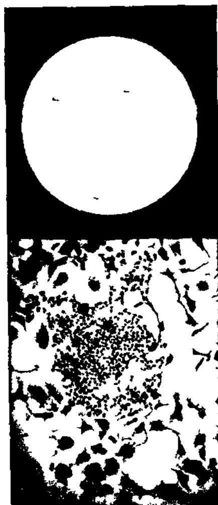


Fig 153 Demonstration of giant production from human cells by x irradiation (*Top*) Plate seeded with single cells and then irradiated with 900 r Only two actual colonies developed The remaining cells became giants each of which is visible to the eye (actual size) (*Bottom*) Photomicrograph of a normal colony growing among the radiation inactivated cells which are forming giants (×75)



Fig 154 Typical colony from the bizarre mutant of S-3 produced as a result of x irradiation Each single cell when plated in nutrient medium produces a colony studded with monster forms as shown

in the mammalian body tissues Under specified conditions single cells may exhibit molecular biochemical requirements for growth which have not been found heretofore in bacteria An example of such action is the need exhibited by single cells of the HeLa S3 clone for cholesterol The quantitativeness of the single-cell plating technic permits titration of cholesterol by a bio-assay with human cells in a manner completely analogous to that used with the B vitamins with bacteria

GENETICS AND THE ACTION OF IONIZING RADIATION

This method has been used to measure the action of ionizing radiations on mammalian cells and has permitted determination of their x ray survival curve as shown in Figure 152 These experiments have yielded values of the mean lethal dose of radiation for various human cells varying between 96 to 160 r a quantity much lower than that which had been presumed to represent the toxic radiation level of x rays for somatic cells Curves obtained for cells from 4 different organs including both normal and neoplastic types are very similar An abundance

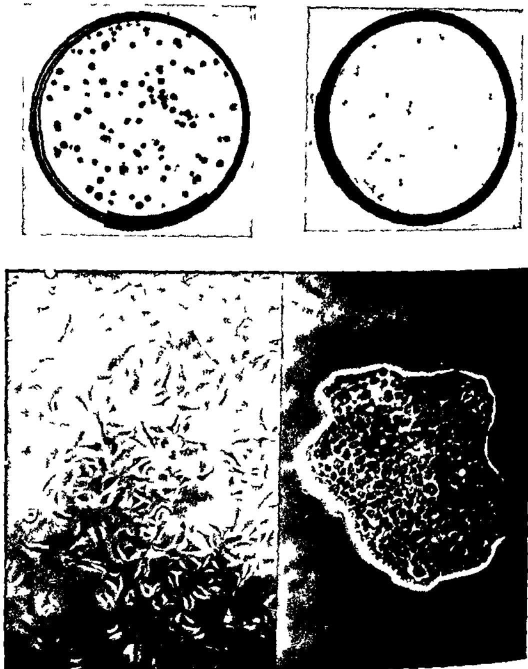


Fig 155 Demonstration of the effect of human serum in producing highly stretched migratory spindle shaped cells instead of compact polygonal epithelial like cells in colonies of S-3 The pair of photographs on the left *(top and bottom)* represent plating of S-3 cells in a medium containing 20 per cent human serum The pair of photographs on the right *(top and bottom)* show the results of plating exactly the same number and kind of cells in a medium containing no human serum but 10 per cent of horse or porcine serum All other conditions were identical in the two platings The photomicrographs represent the appearance of typical colonies taken from each plate

of evidence indicates that the process primarily responsible for the destruction of the cell's reproductive power is a damage to the genetic apparatus

Calculation shows that cell death occurs on the average after absorption of a total quantity of x rays equivalent in energy to a temperature rise of only one ten thousandth of a degree centigrade Hence it is not surprising that these cells whose reproductive powers have been destroyed by x irradiation continue to metabolize abundantly carrying out extensive biosynthesis Such cells continue to grow without reproduction forming giant cells readily visible to the unaided eye (Figs 153 and 154) These giants constitute a fascinating biologic population with many interesting properties including an enormously increased susceptibility to virus attack While these giant cells have never been observed to reproduce they can carry out many specific functions including phagocytosis of vital dyes and specific biosynthesis of many viruses From unirradiated populations and from survivors of irradiated cell inocula new clonal stocks have been isolated with behavior sufficiently different from the wild type to permit their designation as mutants Some of these new stocks display characteristically different growth requirements from the wild type (Fig 155) Others are characterized by different colonial morphologies also shown in Figure 155 Studies aimed at isolating mutant markers useful in genetic investigations are in progress

APPLICATION TO STUDIES OF CELL DIFFERENTIATION

By means of these technics study has been carried out on clonal cell stocks isolated from a variety of normal tissues obtained from a single individual It has proved to be possible to isolate stable lines from the skin which consistently grow only in the fibroblastlike form even after many generations of in vitro cultivation Single lines plated in any medium available if they grow at all invariably produce typical fibroblastlike colonies Other clonal cell lines obtained from the lung are completely epithelial in morphology and equally stable and consistent in the expression of this characteristic Finally clonal cell lines have also been isolated from the lung which were typically fibroblastlike in morphology throughout clonal isolation and for approximately 20 generations of growth thereafter but which then changed their form to an epithelial like morphology

In addition we have found that certain epithelial cells can change their form to simulate a fibroblastic morphology in a completely reversible fashion as a result of addition to the medium of certain fractions of human serum These experiments suggest that the single-cell technic may be helpful in unraveling the wide variety of variation mechanisms available to mammalian cells which undoubtedly play important roles in differentiation

VIRUSLIKE INTERACTION

The use of single-cell plating has been adapted to measurement of the lethal effect of viruses on cell reproduction The ability of a virus particle to destroy a cell's colony forming capacity has been made the basis of a new method for titration of virus particles and for measurement of the process of virus penetration into its host cell Various aliquots of a virus can be added to a series of tubes containing identical cell suspensions After virus attachment the single cells are plated and the colony forming efficiency is scored This method provides a new quantitative assay of a separate virus function

One of the most interesting of our observations made possible by the single-cell plating technic was that mammalian cells can become carriers of an otherwise lethal virus without loss of their ability to multiply indefinitely as apparently healthy cells This state is achieved in a small proportion of the cells when a large population is infected with virus The cells in which this virus-carrier state is achieved continue to reproduce normally and form laboratory stocks which have been stable for more than 2 years or 100 generations When plated as single cells 60 to 70 per cent of this population forms normal macroscopic colonies However when these same cells are plated on top of a layer of x irradiated giant cells which are particularly susceptible to destruction by virus action each carrier cell produces a plaque in which the giants are completely disintegrated A further parallelism between these cells and the lysogenic virus-carrying bacteria lies in their immunity to the destructive action of an inoculum of free virus sufficient to annihilate the parent cell

SUMMARY

The single-cell plating technic makes possible application to mammalian cells of quantitative operations that have proved fruitful in the physiologic and genetic virus studies of microorganisms These tools may provide new approaches to many of the classical biologic problems

BIBLIOGRAPHY

Cieciura S J Marcus P I and Puck T T The use of x irradiated HeLa cell giants to detect latent virus in mammalian cells Virology 3 476 1957

Danford K K Earle W R and Likely E D J Nat Cancer Inst 9 229 1948

Eagle H Nutrition needs of mammalian cells in tissue culture Science 122 501 1955

Earle W R Sanford K K Evans V J Waltz H K and Shannon J P Jr J Nat Cancer Inst 12 133 1951

Evans V J Bryant J C McQuilkin W T Fioramonti M C Sanford K K Westfall B B and Earle W R Cancer Res 16 77 1956

Fisher H W and Puck T T On the functions of x irradiated feeder cells in supporting growth of single mammalian cells Proc. Nat Acad Sc 42 900 1956

Fisher H W Puck T T and Sato G Proc Nat Acad Sc 44 4 1958

Luria S E Animal Virology p 39 New York Wiley 1953

Marcus P I Cieciura S J and Puck T T Clonal growth in vitro of epithelial cells from normal human tissues J Exper Med. 104 615 1956

Puck T T Cieciura S J and Fisher H. W Clonal growth in vitro of fibroblast like cells from normal human tissues J Exper Med. 106 145 1957

Puck T T and Fisher H W Genetics of somatic mammalian cells I Demonstration of the existence of mutants with different growth requirements in a human cancer cell strain HeLa J Exper Med 104 427 1956

Puck T T and Marcus P I A rapid method for viable cell titration and clone production with HeLa cells in tissue culture the use of x irradiated cells to supply conditioning factors Proc Nat Acad Sc 41 432 1955

——— J Exp r Med 103 653 1956

Puck T T Marcus P I and Cieciura S J Clonal growth of mammalian cells in vitro Growth characteristics of colonies from single HeLa cells with and without a "feeder layer J Exper Med 103 273 1956

Todd J C and Sanford A H Clinical Diagnosis by Laboratory Methods p 724 Philadelphia Saunders 1950

Westfall B B Evans V J Peppers E V Hawkins N M and Earle W R Effect of glutamine on growth and metabolism of liver cells in vitro J Nat Cancer Inst 17 131 1956

Alterations in Clonal Populations of Monkey Kidney Cells

DR RAYMOND C PARKER

In recent years numerous reports have concerned the development of strains of altered cells that can be propagated in vitro indefinitely Some of the alterations occurred gradually others suddenly Occasionally they resulted in round cells with a tendency to live and multiply in suspension even in stationary cultures At other times they yielded morphologically uniform populations of cells with a tendency to live and multiply while attached to the glass In most instances the altered cells cannot readily be identified with any cell type existing in the body Fortunately however they usually can be centrifuged washed and propagated by quantitative culture procedures The altered cell strain that is best known is Earle s L strain derived from the subcutaneous connective tissue of a normal C3H mouse Before alteration the cells were cultivated for long periods in plasma clots treated regularly with horse serum and chick embryo extract Eventually some of the substrains were exposed to methylcholanthrene Over a period of 1 to 3 months after the addition of carcinogen to the cultures cells of some of the substrains lost their fibroblastlike appearance became rounded and granular and many giant cells developed Still more interesting several of the altered strains including strain L consistently produced tumors when injected into mice and certain of the control strains also became altered and produced tumors on injection

In previous communications from our laboratory we described cell alterations that have occurred in cultures of normal and malignant cells from many sources including normal kidney epithelium from mouse rat rabbit monkey and man It is the purpose of the present report to review the facts already known about cell alterations in cultures of monkey kidney epithelium and to describe efforts now being made to understand certain chromosomal changes observed in cultures of monkey kidney epithelium both before and after cell alterations have occurred

In 1953 we prepared a series of cultures from chopped monkey kidney and carried them in fluid medium consisting of horse serum chick embryo extract and balanced saline After several weeks great showers of round cells (Fig 156 *top left*) appeared in many of the cultures and when isolated they had a tendency to multiply in suspension even in stationary cultures When they were propagated in tubes rotated at 50 to 60 rpm according to the method of Graham and Siminovitch it was possible to achieve a hundredfold increase in the logarithmic phase of multiplication without an initial lag period During the past 3 years many additional experiments have been made from freshly explanted monkey kidney cultivated in synthetic media supplemented with horse serum In many of these cultures altered cells sometimes appeared as early as 51 days and as late as 107 days after explantation With 2 exceptions in which free living round cells were obtained the altered cells (Fig 156 *top right and bottom left*) remained adherent to the glass of stationary cultures and resembled Earle s L cells In 1 experiment in which some of the cells were subcultured at 7 14 22 37 and 53 days respectively altered cells appeared between the 51st and 60th day after explantation regardless of the number of times they had been subcultured Figure 156 top right shows a nest of altered cells with the unaltered kidney epithelium around the edge Figure 156 bottom left shows altered cells at the upper right and several unaltered epithelial cells at the lower left In another experiment monkey kidney epithelium was kept alive for 6 months in a chemically defined medium (No 858) without protein supplement of any sort but alterations were not obtained Figure 156 bottom right shows the healthy appearance of the epithelial cells at 91 days prior to which the cells had been subcultured twice

Because all of the alterations that have been described up to this point occurred in cultures containing mixed cell populations the possibility was not excluded that they were due to the selec

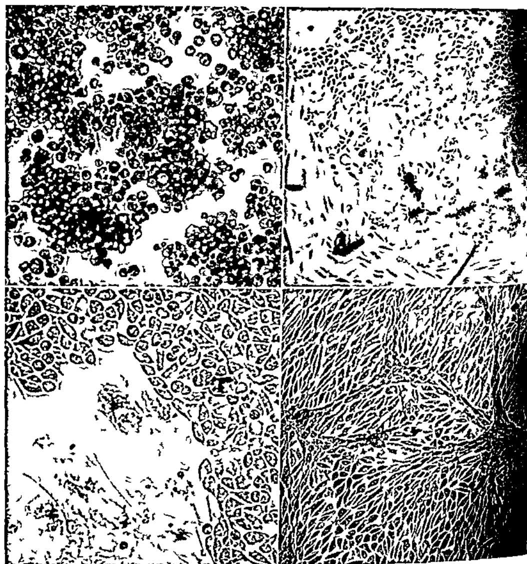


Fig 156 (*Top left*) Altered cells derived from mixed cell culture of monkey kidney tissue after 82 days in 40 per cent horse serum and 3 per cent chick embryo extract in balanced saline Even in stationary cultures these cells tend to multiply while unattached to the glass (Culture 19017) (×180) (*Top right*) Nest of altered cells in 67-day-old culture (20739 10) prepared from freshly explanted monkey kidney tissue and maintained in CMRL-857[16] supplemented with 20 per cent horse serum Note unaltered epithelium surrounding nest of altered cells which contains several giant cells (×38) (*Bottom left*) Altered cells (upper right) and several unaltered epithelial cells (*lower left*) in culture (C181 1) prepared from trypsinized suspension of monkey kidney tissue after 120 days in CMRL-857 supplemented with 20 per cent horse serum during which time it was subcultured once (×200) (*Bottom right*) Unaltered epithelial cells in a culture (C/77) of a series that was prepared from freshly explanted monkey kidney tissue and had been maintained for 91 days (at the time this photograph was made) in unsupplemented chemically defined medium CMRL-858[19] During this period the series had been subcultured twice (×100)

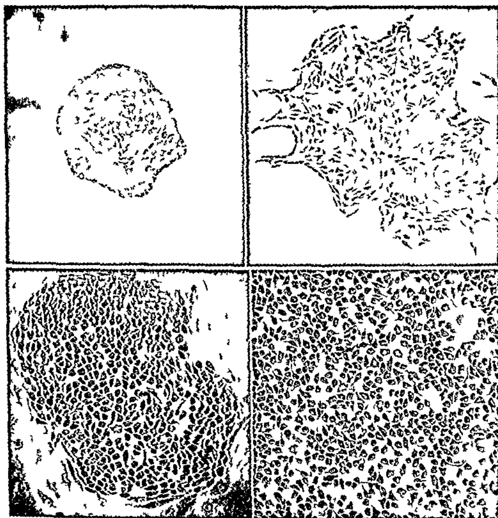


Fig 157 (*Top left*) Clone of monkey kidney epithelial cells 8 days after a single cell had been isolated in a capillary drop of medium on a coverslip under a layer of oil in a Petri dish The medium which was renewed twice during this period was CMRL 1066 supplemented with 20 per cent horse serum (Culture 21798 9) (×38) (*Top right*) Another clone of monkey kidney cells 10 days after a single cell had been isolated under the conditions described for Culture 21798 9 (Culture 21909 2) (×38) (*Bottom left*) Clone of monkey kidney epithelial cells undergoing cellular alteration The clone was left on the coverslip for 13 days then transferred to a 13 × 100 *mm* test tube 16 days before this photograph was made Note unaltered epithelial cells surrounding nest of altered cells (Culture 21877) (×100) (*Bottom right*) Cells from one of several nests of altered cells that developed in the clone shown at the bottom left This subclone (Culture 21936-3) had been maintained for 6 days in a Carrel flask when this photograph was made (×100)

tion of particular cell types present in the original explants To eliminate this possibility it was necessary to observe the phenomenon in clonal populations derived by single-cell isolation Accordingly single cells from cultures of monkey kidney epithelium were isolated in capillary drops of medium on a coverglass under a layer of oil in a 60 mm petri dish *according to a technic devised by deFonbrune* and adapted to animal cell work by Lwoff and collaborators Examples shown are one such clone 8 days after the single cell had been isolated on the coverglass and another clone that was photographed on the tenth day (Fig 157 *top left and right*) Both consist of typical epithelium The medium which consisted of CMRL-1066 supplemented with 20 per cent horse serum was always passed through fritted glass filters after the addition of the serum The developing clones were left in the capillary drops with 2 changes of medium a week until they were large enough to be transferred to flasks or tubes By these means then 350 clones have been developed that survived for a week or more Of these 350 clones 27 per cent survived for at least 1 month 12 per cent for at least 2 months and 6 per cent for at least 3 months Altered cell strains (Fig 157 *bottom left and right*) were derived from 21 of these clones and the average time required for the alterations to occur was 33 days after the single cell isolation or 41 days after explantation from the monkey The most rapid cell alteration occurred 19 days after the single-cell isolation or 29 days after explantation from the monkey The slowest alteration occurred 81 days after explantation These experiments proved conclusively that the appearance of the new cell type was not due to the selection of a particular cell explanted from the organism Appropriate care was always taken of course to eliminate the possibility of contaminating the clones with cells from other sources

From time to time over the past few years, various strains of altered monkey kidney cells derived from mixed cell populations have been tested for polio virus susceptibility but always with negative results It could not have been known in any of these experiments whether or not the immediate progenitors of the altered



Fig 158 (*Left*) Unaltered monkey kidney epithelial cells in culture shown in Figure 157 bottom left photographed 4 days after infection with polio virus The cells are completely destroyed (×112) (*Center*) Altered cells in culture shown in Figure 157 bottom left photographed 4 days after infection with polio virus The cells are not visibly affected by the virus (×112) (*Right*) Photomicrograph of a typical metaphase plate (diploid) of an unaltered cell in a culture of monkey kidney epithelium (×1 125)

cells would have b en susceptible but once alterations were obtained in developing clones it became possible to study the direct effect of the alteration process on virus susceptibility In an experiment of this sort a newly established clone developed as epithelium On the 13th day when a fair sized sheet of cells had formed on the coverglass they were detached with trypsin and transferred to a small stationary test tube where the survivors continued to multiply slowly to form epithelial sheets as before Then 16 days later several nests of exceedingly active altered cells (Fig 157 *bottom left*) appeared in the epithelium and cells from one of these nests (such as you see here) were removed with a capillary pipette and transferred to a larger container where they continued to multiply with great rapidity (Fig 157 *bottom right*) as you see here but the transforming culture itself which contained both the altered and the unaltered cells was then treated with the MEF_1 strain of polio virus at a concentration of 2×10^4 plaque forming particles per ml After 7 days the virus had severely damaged the unaltered epithelial cells without visibly affecting the altered cells By 4 days when the unaltered cells were completely destroyed (Fig 158 *left*) the altered cells (Fig 158 *center*) still appeared to be healthy and active This experiment proved conclusively that the change in susceptibility of the population to polio virus occurred during alteration

It might be helpful at this point to summarize the information that has been obtained by observing cell alterations many times in cultures of monkey kidney epithelium Thus it is known that

1 The appearance of altered cells in a culture population is not due to the selective advantage of a particular cell type present in the original explants

2 Altered cells from monkey kidney differ morphologically from the parent-cell type but are remarkably similar morphologically to altered cells from other sources

3 Altered cells may appear in a healthy young population or in an old one in which multiplication of the parent-cell type has ceased They continue to multiply indefinitely even in cultures in which the parent-cell type disappears They are capable of unlimited survival

4 Altered cells may appear in relatively small populations One clone became altered on the 26th day when there were only 30 surviving cells

5 Altered cells from monkey kidney have never been observed to revert to the parent-cell type Of course this stability could be due to the selective advantage of the altered cells

6 Altered cells may be propagated continuously under conditions that are not favorable to unaltered cells Thus altered cells from monkey kidney epithelium unlike the parent-cell type may be propagated in agitated suspensions

7 Altered cells from monkey kidney epithelium unlike freshly isolated monkey kidney epithelium are either not susceptible or only slightly so to polio virus Also it has been found by Drs E S Lennox and A S Kaplan of the University of Illinois that altered cells from monkey kidney cultures are less sensitive to diphtheria toxin than are the unaltered cells

Altered cells from monkey kidney epithelium differ from unaltered monkey kidney cells both in chromosome number and in chromosome morphology Unaltered monkey kidney cells have 42 chromosomes all of which are metacentric and most of which can be identified individually Figure 158 right shows a normal diploid A number of clones and subclones of altered monkey kidney cells were examined by the colchicine-orcein technic which will be described in more detail later The number of chromosomes in each of 100 nuclei of one such monkey clone are shown in Figure 159 where

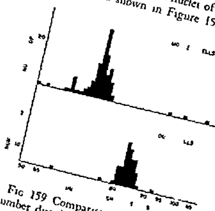


Fig 159 Comparison of chromosome number distribution in altered cells derived from monkey and mouth kidney epithelium

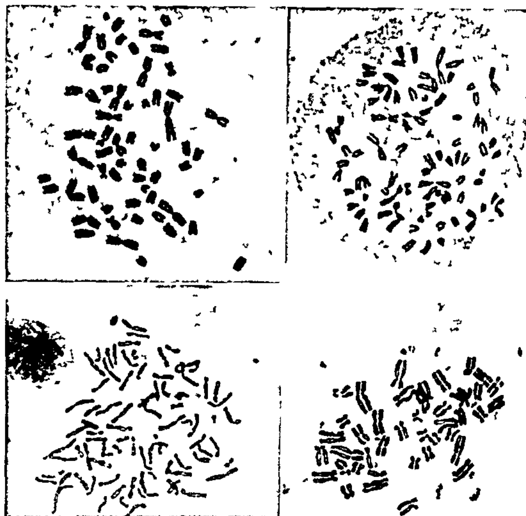


FIG 160 (*Top left*) Photomicrograph of a typical metaphase plate of an altered cell from a clone (2 2) derived from monkey kidney epithelium (×1 000) (*Top right*) Photomicrograph of a typical metaphase plate of an altered cell from a culture strain derived from mouse kidney epithelium (×1 000) (*Bottom left*) Photomicrograph of a typical metaphase plate (tetraploid) of an unaltered cell in a culture of monkey kidney epithelium (×1 000) (*Bottom right*) Photomicrograph of a 4 stranded tetraploid metaphase plate of an unaltered cell in a culture of monkey kidney epithelium (×1 000)

it will be seen that the distribution is essentially unimodal with a peak at 69 chromosomes Other clones that were examined showed a similar distribution of chromosome numbers A typical altered chromosome complement is shown in Figure 160 top left where 18 of the chromosomes are metacentric the remainder acrocentric In clear preparations an extremely short arm can always be seen in chromosomes of this type the centromeres are not strictly terminal Table 104 shows a more refined analysis of a subclone in that it gives the frequency distribution of the number of metacentrics in nuclei of a given chromosome number Thus, for example of 17 nuclei with a total 69 chromosomes 4 had 17 metacentrics 9 had 18 metacentrics 3 had 20 and 1 had 21 This information illustrates something of the heterogeneity that one obtains even in cultures arising from a single cell When other clones and subclones were ana

TABLE 104 DISTRIBUTION OF METACENTRICS IN NUCLEI OF DIFFERENT CHROMOSOME NUMBER (MONKEY KIDNEY CLONE 2 2)

TOTAL NUMBER OF CHROMOSOMES	NUMBER OF METACENTRICS 15	16	17	18	19	20	21
59		1					
60				1			
61			3				
62			1	1	1		
63				4	1		
64	1		3	3	1		
65			4	2			
66			5	1			
67		2	4	7	4		
68			3	10	1	1	
69			4	9		3	1
70		1	1	5	2	7	
71			1			1	

lyzed the frequency distribution of the number of metacentrics was similar to those found in this clone Also when we examine the distribution of total chromosome numbers in a typical subclone we find that the mode and the range of chromosome numbers is similar to those of the parent clone When the cytologic analysis was refined still further certain marker chromosomes could be distinguished among the metacentrics and the acrocentrics but even with respect to these marker chromosomes all clones and subclones that were examined were remarkably similar

The results of these studies have indicated that all carefully examined clones and subclones are identical with respect to modal chromosome number approximate range of chromosome numbers morphology of chromosomes and the presence of distinctive marker chromosomes This essential identity of chromosome pattern rules out the possibility that altered cells arise from random breaks and reunions of chromosome fragments even if one grants the unlikely assumption that this particular alteration confers a strong selective advantage We are left then with only two possibilities either we are dealing with a directed induced chromosome change of a kind and specificity so far unknown or cellular contaminations at some level have been responsible for the similarity of these clones Though we have been aware of the danger of cellular contaminations throughout all of our work we cannot at this moment exclude it as a possibility These studies were made in collaboration with Drs L Siminovitch Department of Microbiology School of Hygiene and K H Rothfels Department of Botany

Because of simultaneous studies made by other colleagues in Toronto namely Drs A A Axelrad and E A McCulloch of the Ontario Cancer Institute it is possible to compare the chromosome changes associated with alterations in monkey kidney cultures with those that occur in cultures from the mouse Newly established cultures of kidney epithelium from CF1 mice were found to have a chromosome complement similar to that described for normal diploid mouse cells The vast majority of cells had 40 chromosomes all of which were acrocentric But 3 independent strains of altered mouse cells were found to be similar to each other and distinct from altered monkey kidney cells in that the modal chromosome number was 83 of which 15 to 17 were metacentric A typical altered cell complement is shown in Figure 160 top right Figure 159 is a histogram of one of the altered mouse strains (below) shown in comparison with that of an altered monkey strain (above) Within the last few weeks my colleagues in Toronto have found still another chromosome picture associated with alteration in a culture of kidney cells from a 20-day-old chick embryo In this case the modal chromosome number was 62 to 64 with 11 to 14 metacentrics Up to the present then we have encountered 3 distinct categories of altered cells with respect to their chromosome complement (1) the monkey kidney type (2) the mouse kidney type and (3) the chick kidney type It should also be mentioned that Levan has reported changes in chromosome number and morphology in 2 of the human strains of cells developed by Chang

The alterations that have been described occurred only after prolonged cultivation of the cells It could not have been known whether the chromosome changes occurred at the time of alteration or that transitional changes may have occurred earlier Accordingly and again in collaboration with Dr Siminovitch and Dr Rothfels an effort was made to follow the chromosome constitution of monkey kidney cells as soon as possible after explantation from the animal While no striking changes were seen that could

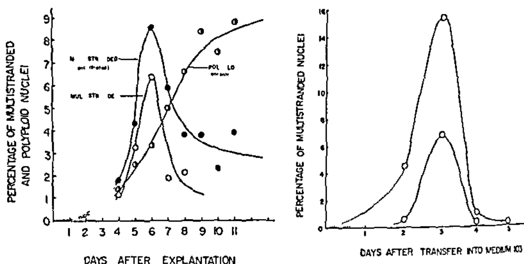


FIG 161 (*Left*) Frequency of multistranded and polyploid nuclei in unaltered monkey kidney cultures as a function of time (*Right*) Frequency of multistranded nuclei in altered monkey kidney cultures as a function of time (data from two separate experiments)

be related directly to alteration we did observe interesting chromosome irregularities that I shall now describe Apart from dividing cells with the normal diploid complement of 42 chromosomes a large number of nuclei showed other types of division figures including tetraploid nuclei (one of which is shown in Fig 160 *bottom left*) and nuclei similar to those observed by Levan and Hauschka in mouse ascites tumors and described by them as endoreduplicated metaphases Because of the uncertainty as to the origin of these forms in our material we shall refer to them only as multistranded nuclei A 4 stranded tetraploid is shown in Figure 160 bottom right We have also observed 4 stranded nuclei of even higher ploidy as for example 8 stranded octoploids

In a series of experiments designed to study the appearance and the fate of these unusual division figures as a function of time freshly isolated kidney tissue was trypsinized and cell suspensions were placed for incubation in 100 petri dishes that were maintained in a controlled atmosphere to prevent evaporation and loss of CO Each dish contained 2 microscope slides upon which cells would settle and proceed to multiply In order to have slides for study over an extended period as many as 100 cultures were prepared from a single pooled suspension and since preliminary studies had indicated that the size of the inoculum might be important, 2 concentrations of cells were used that differed by a factor of 4 The medium consisted either of CMRL-1066 supplemented with 20 per cent horse serum or one comprised of calf serum lactalbumin hydrolysate yeast extract Tris buffer and inorganic salts which we shall refer to as medium 103 On the second day the cultures were washed and the medium renewed thereafter the medium was renewed daily On the third day and at daily intervals until the end of the experiment slides were removed from the dishes treated with a hypotonic saline solution for 30 minutes to spread the chromosomes fixed for 5 minutes in acetic alcohol dried in air and stained with aceto-orcein The preparations could be studied as temporary mounts and if necessary could be made permanent by the CO ice method of Conger and Fairchild For each slide that was scored about 1 000 cells in metaphase were completely classified as to ploidy and strandedness This classification was not based on detailed chromosome counts in all cases Thus for example if the chromosome mass suggested 42 chromosomes the nucleus was scored as diploid if the mass seemed twice the size it was scored as tetraploid

The results of a typical experiment of this

sort are shown in Figure 161 left It will be seen that both the dilute and the concentrated cell suspensions yielded cultures in which the percentage of multistranded nuclei was low at first but rose rapidly to a peak at about 6 days In some experiments the percentage of multistranded forms reached values of up to 20 per cent Beyond 6 to 7 days the percentage of multistranded nuclei decreased and remained low for the duration of the experiment but the highest percentage of multistranded nuclei was always found in cultures initiated from the more concentrated cell suspensions During the early days the percentage of polyploids always lagged behind that of the 4 stranded forms but increased later to about 10 per cent of the metaphase nuclei Because these data are preliminary it is perhaps premature to draw definite conclusions as to the induction and the disappearance of multistranded nuclei in cultures of unaltered monkey kidney epithelium At least 2 possibilities come to mind namely that certain cells of the intact kidney may be predisposed to this condition and cultivation in vitro allows the character to be expressed or that the shock of explantation and subsequent cultivation may in itself induce the formation of multistranded nuclei in a certain percentage of the dividing cells

I shall now describe similar results that were obtained in experiments with altered monkey kidney cells for they clarify to some extent the interpretation of the earlier experiments It was first found that altered monkey kidney cells under certain conditions also yielded multistranded nuclei an example of which is shown in Figure 162 In experiments designed to study more carefully the conditions under which multistranded nuclei could be obtained the altered cells were propagated in suspension in roller tubes in medium CMPL-1066 supplemented with 20 per cent horse serum From these suspensions petri dish cultures were prepared as before in another medium No 103 and colchicine was added about 18 hours before the slides were removed for staining As in the earlier experiments a daily estimate of the percentage of multistranded nuclei was made from the third day In Figure 161 right which includes data from 2 experiments the results are strikingly similar to those obtained with unaltered cells except that the highest percentage of multistranded forms occurred somewhat earlier at 2



Fig 162 Photomicrograph of a metaphase plate of an altered cell from a clone (2 ?) derived from monkey kidney epithelium Note the 4 stranded condition of the chromosomes to give twice the basic chromosome number (×1 000)

to 4 days This effect was found in each of several experiments though the magnitude of the peak values varied and was sometimes as high as 30 per cent However when cells propagated in suspension in CMPL-1066 supplemented with horse serum were transferred to petri dishes containing the same medium rather than 103 few multistranded nuclei were found Also when the reverse transfer was made for example when cells propagated in medium 103 were transferred to petri dishes containing supplemented 1066 little or no induction of multistranded nuclei occurred.

These results indicate that it is not the change of medium per se that is responsible for the appearance of multistranded nuclei but rather that the initiation of cell division in medium 103 stimulated in some way the appearance of these nuclei Of course these observations do not suggest that these are the only conditions that might effect their induction In fact in preliminary experiments we have found that altered cells that were propagated in petri dishes in 103 for more than 5 days then trypsinized and replated in 103 also yielded elevated percentages of multistranded nuclei. Similar results have been observed occasionally when unaltered cells grown in petri dishes in 103 were trypsinized and replated in 103 Also large percentages

of multistranded nuclei have occasionally been found even without medium 103 in cultures in which unaltered cells were placed immediately after explantation in supplemented 1066

The experiments with altered cells would seem to indicate that the induction of multistranded nuclei can occur in the absence of genetic predisposition since the altered cells used for these experiments were derived by single-cell isolation It would seem more plausible to suggest that the multistranded forms arise as the result of some sort of shock both in unaltered and altered cell material

Comparison of the rates of formation of multistranded and polyploid nuclei in the unaltered cell cultures indicates that the former appear before the latter and that as time goes on the multistranded forms tend to disappear whereas the polyploids remain Although the extent of polyploidy was not scored carefully in the experiments with altered cells cultures that had previously shown a large percentage of multistranded nuclei later showed a large percentage of polyploids These results show quite clearly then that in our material polyploid forms are derived from multistranded nuclei To this extent our results support the similar conclusion of Levan and Hauschka

BIBLIOGRAPHY

Berman L and Stulberg C S Eight culture strains (Detroit) of human epithelial like cells Proc Soc Exper Biol & Med 92 730 1956

Chang R S Continuous subcultivation of epithelial like cells from normal human tissues Proc Soc Exper Biol & Med 87 440 1954

Conger A D and Fairchild L M A quick freeze method for making smear slides permanent Stain Techn 28 281 1953

deFonbrune P Technique de Micromanipulation Paris Masson 1949

Eagle H Propagation in a fluid medium of a human epidermoid carcinoma strain KB Proc Soc Exper Biol & Med 89 362 1955

Earle W R Production of malignancy in vitro IV The mouse fibroblast cultures and changes seen in the living cells J Nat Cancer Inst 4 165 1943

Earle W R and Nettleship A Production of malignancy in vitro V Results of injections of cultures into mice J Nat Cancer Inst 4 213 1943

Evans V J Earle W R Wilson E P Waltz H K and Mackey C J The growth in vitro of massive cultures of liver cells J Nat Cancer Inst 12 1245 1952

Firor W M and Gey G O Observations on the conversion of normal into malignant cells, Ann Surg 121 700 1945

Gey G O Some aspects of the constitution and behavior of normal and malignant cells maintained in continuous culture Harvey Lect 50 154 1954 55

Goldblatt H and Cameron G Induced malignancy in cells from rat myocardium subjected to intermittent anaerobiosis during propagation in vitro J Exper Med 97 5 1953

Graham A F and Siminovitch L Proliferation of monkey kidney cells in rotating cultures Proc Soc Exper Biol & Med 89 326, 1955

Healy G M Fisher D C and Parker R C Nutrition of animal cells in tissue culture X Synthetic medium No 858 Proc Soc Exper Biol & Med 89 71 1955

Hull R N Cherry W R and Johnson I S The adaptation and maintenance of mammalian cells to continuous growth in tissue culture Anat Rec 124 490 1956

Jordon W S Human nasal cells in continuous culture I Establishment of two lines of epithelial like cells Proc Soc Exper Biol & Med 92 867 1956

Lennox E S and Kaplan A S Personal communication

Leslie I Fulton W C and Sinclair R Biochemical tests for malignancy applied to a new strain of human cells Nature 178 1179 1956

Levan A Chromosome studies on some human tumors and tissues of normal origin grown in vivo and in vitro at the Sloan Kettering Institute Cancer 9 648 1956

Levan A and Hauschka T S Endomitotic reduplication mechanisms in ascites tumors of the mouse J Nat Cancer Inst 14 1 1953

Lwoff A Dulbecco R Vogt M and Lwoff M Kinetics of the release of poliomyelitis virus from single cells Virology 1 128 1955

McCulloch E A and Parker R C Continuous cultivation of cells of hemic origin in Begg R W (ed) Canadian Cancer Conference vol 2 p 152 New York Acad Press, 1957

Parker R C Cultivation of tumor cells in vitro *in* Begg R W (ed) Canadian Cancer Con

ference vol 1 p 4? New York Acad Press 1955

Parker R C Castor L N and McCulloch E A Altered cell strains in continuous culture a general survey *in* Cellular Biology Nucleic Acids and Viruses (Special Publication) vol 5 p 303 New York Acad Sc 1957

Perry V P Evans V J Earle W R Hyatt G W and Bedell W C Long term tissue culture of human skin Am J Hyg 63 5? 1956

Scherer W F Comparative susceptibility of cells of the same type to infection by poliomyelitis virus Ann New York Acad Sc 61 806 1955

TABLE 107 ANAEROBIC GLYCOLYSIS IN NORMAL MALIGNANT AND TRANSFORMED CELL LINES IN TISSUE CULTURE

		NORMAL (FIBROBLASTS)	MALIGNANT (HEP No 2)	TRANSFORMED (CHANG S LINES)
$Q^{N}_{CO_2}$	Exp 1	16	54	40
	Exp 2	9	37	69

Q   = μl of CO /hr /mg d y w ight, o r ct d f CO tent on in ti ue-cultur media v th um

Leslie Fulton and Sinclair in a metabolic study of an altered cell line derived from normal human embryonic livers found that the glucose consumption of their 3 morphologically normal cell lines recently isolated from embryonic skin kidney and lung were closely similar whereas their morphologically different cell line resembled that of the 3 human carcinomas studied Metabolic studies along these lines are just beginning and high hopes may be entertained for them to be of considerable help in following the change from normal to malignant

Nutrition If normal and known cancer cell lines had different nutritional requirements it might be possible to use this as a criterion for malignancy Although cells kept in continuous culture are known to change their nutritional habits as yet it is impossible to distinguish them on this basis Chang noted that during the isolation of conjunctival liver kidney and appendical cell lines from normal individuals their sensitivity to human sera was quite different Later this sensitivity disappeared Puck and his group in studies already mentioned found that nutritional requirements of the Chang cell lines were identical to the S3 line of HeLa cells On the other hand Parker has noted that monkey kidney cultures kept in chemically defined media without a protein supplement have failed to show altered cells in 3 months and that the cells remained in good condition This finding may be a hint on how to keep our normal cells normal

CHANGES IN REACTIVITY

When a cell line changes in morphology one might expect it to change in either its nutritional or biochemical requirements or in its viral susceptibilities Eagle has pointed out that a strain of HeLa which assumed a fibroblastic form concomitantly showed a biochemical alteration A loss in cytopathogenicity of polio virus for altered cell lines has been reported by numerous workers In addition Bang and Gey had observed that Eastern equine encephalitis selectively destroyed the cells of a derived rat sarcoma their T 333 and had no effect whatsoever on the parent fibroblast However due to the great variety of cytopathogenic effects that one observes when a number of viruses are screened against a number of cell lines it seems unlikely that this method could be used to determine whether a given cell line is cancerous or not Of course if a cell line becomes resistant or susceptible to a certain virus or chemical one could use this as an indication that a change of some sort had taken place

TRANSPLANTATION

The ability of cultured cells to produce tumors in the homologous strain of animal has long been a criterion for neoplasia In the animal studies with rodent fibroblasts which already have been cited cell lines became capable of producing tumors which on histologic examination were sarcomas and could be transferred to other animals of the same strain indefinitely It is impossible to study human altered cell lines in exactly this same way but they can be tested for their ability to produce tumors by implantations into cortisone treated and irradiated weanling rats and also into volunteer cancer patients and normal volunteers Before presenting the results it is necessary to discuss what is meant by a positive or a negative result and here of course there is considerable disagreement It seems that there is no absolute criterion for calling a tumor a cancer although the diagnosis is made every day The pathologist makes up his mind on the basis of clinical findings cell

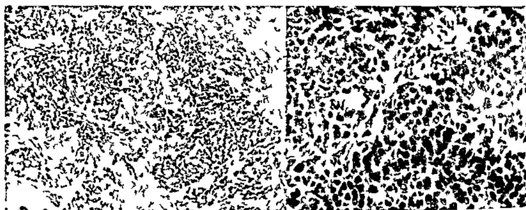


FIG 166 (*Left*) Tumor produced by the Chang conjunctival cells 53 days after the first biopsy and 65 days after inoculation (×46) (*Right*) Same section (×145)

appearance and a vast amount of experience Sections of the tumors produced by our inoculations of the Chang cell lines were reviewed by 4 pathologists and were found to contain cells which looked like epithelial cells with certain characteristics that are associated with neoplasia and if they had been submitted for diagnosis would have been called cancer It immediately is recognized that this is insufficient evidence for calling these particular cell lines cancerous because cancer is regarded as a progressive growth which leads to the death of the host animal and this is certainly not the case here Keeping this in mind a positive result is defined as the appearance at the site of tumor implantation of a nodule 7 to 14 days postinoculation with no sign of a nodule during the intervening time which when removed by biopsy shows epithelial cells in an array and of a type suggesting neoplastic growth

Very briefly in the animal transplantation studies cited here the Chang cell lines were inoculated in quantities of 1 to 4 million subcutaneously into the irradiated and cortisone treated rats in 25 instances and tumors were produced in 13 which contained epithelial cells and were considered to be positive Known cancer cell lines almost invariably produced tumors so there is no doubt that the Chang cell lines are much less vigorous in this regard Control inoculations of cell suspensions of human testicle (4 attempts) placenta (2 attempts) and monkey kidney (1 attempt) failed to produce tumors as did a variety of tissue minces from normal lung and breast This is in contrast with the experiences of Dr Cornell who obtained the same type and number of tumors from monkey and human kidney cell suspensions as he did from the Chang cell lines He pointed out that these all regress in contrast with HeLa the only human cell line studied In turn his findings differ from those of Dr Ginsberg who studied lines of cells derived from normal human tonsillar and adenoidal epithelium which in quite a short period of cultivation (about 6 weeks) showed an al ra tion When inoculated into irradiated and cortisone treated rats 85 to 100 per cent developed tumors which invaded muscle and submaxillary gland and by these criteria were much more malignant than the HeLa strain which he used as a control

Probably there are adequate explanations for difference in results obtained For example as Ginsberg pointed out his strain of HeLa might not have been as invasive as that of Cornell and it may be that the normal cell suspensions with which we failed to produce tumors w e not of good enough quality or were in insufficient numbers

We have also used human volunteers both with and without cancer for implantation experiments by inoculation of 1 to 5 million cells subcutaneously into the flexor surface of the forearm where no lesions were present Tattoo marks were placed directly over the implantation site and excisional biopsies were done whenever nodules become palpable—usually 9 to 14 days after inoculation Two types of controls were used for these experiments human

embryonic and adult fibroblasts which had shown no morphologic change since they were isolated and known cancer cell lines. In the cancer patients (Table 108) 13 implantations of Chang's cells were made; 7 were biopsied and contained epithelial cells and were considered as positive according to our definition; 3 had no palpable nodules and therefore were considered clear-cut negatives and 3 had nodules which regressed. Nineteen cancer patients were inoculated with cancer cell lines; 15 biopsies were done and 14 contained cancer cells; in 2 nothing could be palpated so no biopsy was done and in 2 instances the nodules were allowed to regress. Whenever possible the biopsy sites were rebiopsied at postmortem and in 1 instance of 3 with the Chang cells and 2 of the 3 with the human cancer cell lines tumor cells were found. Figure 166 left shows the histologic appearance of the Chang conjunctival cell line 53 days after the first biopsy and 63 days after implantation. The cells are grouped in columns and cords embedded in connective tissue. Higher power (Fig. 166 *right*) shows that the cells are epithelial in type with very little sign of degeneration. No tumors were formed from the 3 human fibroblast implants. It should be pointed out that there is a great amount of individual variation in the cancer volunteers' resistance to inoculation of either the Chang cell lines or the known cancer lines. For instance, both positive rebiopsies of the tumor cell lines occurred in 1 patient whose susceptibility to cultured cells is attested to by the fact that all implants have taken and at autopsy a metastatic node was found from a chorio-allantoic membrane grown HEP No. 3.

TABLE 108. RESULTS OF INOCULATIONS OF CELL LINES INTO VOLUNTEER CANCER PATIENTS

	CHANG CELL LINES	CANCER CELL LINES
Number of implants	13	19
Positive biopsies	*7†	14/15†
Nothing to biopsy	3	2
Nodules which regressed	3	2
Positive rebiopsies	1/3†	2/3†

* [illegible] 9 [illegible] 14 [illegible] implant[illegible]
† [illegible] the number [illegible] the number of biopsies performed.

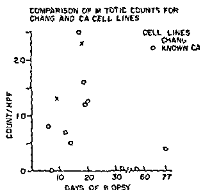


FIG. 167. Average number of mitotic figures per high power field (average of 20) of the tumors produced by known cancer cell lines and the Chang cell lines.

Unfortunately one cannot compare directly the malignancy of the Chang cell lines and the known tumor cell lines. Although it is possible to note such things as degenerating cells, host reaction, and so forth, all of these measurements are subject to error. One might prognosticate that one tumor would regress or continue to grow, but there is no way of proving it. It is possible to obtain a general idea of the activity of growth by doing mitotic counts, and this was done for both groups of tissue by noting the number of mitotic figures seen in an average of 20 fields containing good tumor tissue. Figure 167 shows that there is great variety in the number of mitotic figures in both types of tissue, some of Chang's cell lines having as high an index as the known cancer cell lines and some of the latter being singularly lacking in mitotic activity.

In the normal human volunteers receiving known cancer cell lines, 4 of 10 implantation sites were found to contain cancer cell lines on biopsy 14 days later. However, I do want to emphasize that these cells which were found were quite different from those that we find in the cancer patients in that there was a lot of reaction and the cells were mostly degenerating. No tumors formed in these normal volunteers when the Chang cell lines (3 implants), the HeLa cell lines (2 implants) or human fibroblasts (3 implants) were inoculated.

In addition to the Chang cell lines, we have done some limited studies on the H strain of

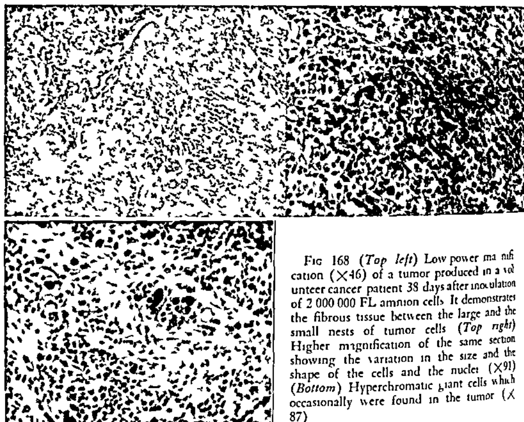


Fig 168 (*Top left*) Low power magnification (×46) of a tumor produced in a volunteer cancer patient 38 days after inoculation of 2 000 000 FL amnion cells It demonstrates the fibrous tissue between the large and the small nests of tumor cells (*Top right*) Higher magnification of the same section showing the variation in the size and the shape of the cells and the nuclei (×91) (*Bottom*) Hyperchromatic giant cells which occasionally were found in the tumor (× 87)

amnion which was obtained through the courtesy of Dr Jorgen Fogh This cell strain originally derived from human amnion had had 40 trypsinizations when we received it Two million cells were implanted into 2 sites in the forearm of a volunteer patient who had reticulum cell sarcoma A tumor appeared in both sites 1 was biopsied 14 days later while the other was allowed to remain in situ where it continued to grow slowly until the death of the patient from his own disease 38 days later Figure 168 shows the microscopic appearance of the tumors Dr Stephen S Sternberg who examined these sections gave the following report

Sections reveal tissue having all the morphological criteria of a malignant tumor The growth pattern shows considerable variation in different areas A major portion of the tumor is growing in solid masses in some portions in small nests Both connective tissue and adipose tissue are involved The cells show moderate variation in size and shape There is nuclear irregularity and hyperchromatism Many cells have enlarged dark and prominent nucle[i] The cytoplasm is abundant and generally p[...] and either homogeneous or granula[r] In so[me] cells the cytoplasm is vacuolated and in oth[er] mucus like secretions appear to be present Oc[ca]sional tumor giant cells are present

Chromosome studies were also conducted Dr A Levan and Dr J Biesele who found t[he] chromosome number varied around 80 and th[at] many chromosomes were abnormal

DISCUSSION

It is apparent that we do have methods a[nd] technics by which cells grown in tissue cultu[re] can be characterized It seems clear that judgment of the cell status cannot be made o[n] one test alone but if in a number of tests a ce[ll] line seems to resemble the malignant prototy[pe] more than the normal one is justified in doub[t]ing its claim to normality This does not mea[n] that it can be said to have become maligna[nt] even though it is capable of producing tumo[rs] which ordinarily would be termed malignan[t]

since the clinical criteria of lethality and metastases have not been fulfilled

The point which I believe needs emphasis is that cells derived from normal sources and maintained in tissue culture should be watched constantly and their characteristics determined so that when they are referred to as normal we may have some assurance that they do have the morphologic metabolic and growth characteristics that we associate with normal cells

BIBLIOGRAPHY

Bang F and Gey G Viruses and cells a study of tissue culture applications Tr New York Acad Sc 13 324 1951

Perlman L and Ginsberg C S Eight culture strains (Detroit) of human epithelial like cells Proc Soc Exper Biol & Med 92 730 1956

Chang R S Continuous subcultivation of epithelial like cells from normal human tissues Proc Soc Exper Biol & Med 87 440 1954

—— Indirect evidence of changes in nutrient requirements of human epithelial like cells in continuous culture *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 315 320 New York New York Acad Sc 1957

Cornell L L McAllister R M and Wagner B W Criteria for determining malignancy in tissue culture cell lines in the albino rat *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 341 350 New York New York Acad Sc 1957

Eagle H Discussion *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) p 299 New York New York Acad Sc 1957

Earle W H and Nettleship A Production of malignancy in vitro V Results of injection of cultures into mice J Nat Cancer Inst 4 213 1943

Fogh J and Lund R O Continuous cultivation of epithelial cell strain (FL) from human amniotic membrane Proc Soc Exper Biol & Med 94 532 1957

Gey G O Some aspects of the constitution and behavior of normal and malignant cells maintained in continuous tissue culture *in* The Harvey Lectures p 154 New York Acad Press 1954 1955

Ginsberg H S Discussion of papers presented under the title Properties acquired by cells maintained in continuous culture *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 362 363 New York New York Acad Sc 1957

Haff R F and Swim H E Serial propagation of three strains of rabbit fibroblasts their susceptibility to infection with vaccinia virus Proc Soc Exper Biol & Med 93 200 1956

Henle W Personal communication

Jordan W E Human nasal cells in continuous culture establishment of two lines of epithelial like cells Proc Soc Exper Biol & Med 92 867 1956

Leighton J Kline I and Orr H C Transformation of normal human fibroblasts into histologically malignant tissue in vitro Science 123 502 1956

Leslie I Fulton W C and Sinclair P Biochemical tests for malignancy applied to a new strain of human cells Nature 178 1179 1956

Levan A Chromosome studies on some human tumors and tissues of normal origin grown in vivo and in vitro at the Sloan Kettering Institute Cancer 9 648 1956

Marcus P I Cieciura S J and Puck T T Clonal growth in vitro of epithelial cells from normal human tissues J Exper Med 104 615 1956

Moore A E Biological consideration of normal and abnormal cell lines Tr New York Acad Sc 19 435 1957

—— Oncolytic properties of viruses Texas Rep Biol & Med 15 588 1957

—— Tumor formation by cultured cells derived from normal and cancerous tissues *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 321 323 New York New York Acad Sc 1957

Moore A E Southam C M and Sternberg S S Neoplastic changes developing in epithelial cell lines derived from normal persons Science 124 127 1956

Parker R C Castor L N and McCulloch E A Altered cell strains in continuous culture a general survey *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 303 313 New York New York Acad Sc 1957

Scherer W F Comparative susceptibility of cells of the same type to infection by poliomyelitis virus Ann New York Acad Sc 61 806 1955

Southam C M Moore A E and Rhoads C P Homotransplantation of human cell lines Science 125 158 1957

Swim H E and Parker R F Alterations in the nutritional requirements of mammalian

fibroblasts in response to changes in the composition of the medium *in* Cellular Biology, Nucleic Acids and Viruses (Special Pub No 5) pp 351 355 New York New York Acad Sc 1957

Syverton J T Comparative studies of normal and malignant human cells in continuous culture *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 331 340 New York New York Acad Sc 1957

Toolan H W Growth of human tumors in cortisone treated laboratory animals the possibility of obtaining permanently transplantable tumors Cancer Res 13 389 1953

DISCUSSION

DR SYVERTON Drs Eagle Puck Parker and Moore in these 4 excellent papers have exemplified the state of sophistication reached by animal cell culture Time is inadequate to consider implications of these papers save for one point The conjunction of subject matter perhaps inadvertent emphasizes a problem facing all who work with established strains of animal cells in virology Several years ago we foresaw need for mammalian cell strains in continuous culture for poliomyelitis research The cells developed were obviously potentially valuable for vaccine production but at first the cell strains were of malignant origin So there appeared what *Westwood and his associates aptly termed the* "specter of malignancy of cultivated cells Specters are notoriously tenuous and pervasive This one no exception has gotten mixed up with most of the changes we see in mammalian cells as part of the normal spectrum during serial cultivation The 4 papers indicate dimensions of the problem and suggest means of attack

I suggest that we as virologists keep our objectives firmly in mind The cancer researcher is interested in cell transformations pertaining to malignancy as virologists we are interested in cell transformations pertaining to normality If fear of malignancy should prevent application of advanced cell technic to preparation of vaccines protective against old and new virus diseases that will be a consequence serious enough failure to understand the bases of departure from normality on the part of cultivated cells will be tragic since it will prevent us from translating cell-culture studies into terms of disease in vivo

I believe that these dangers can be avoided if we will concentrate on investigation rather than speculation It is true as Earle Gey and others have shown that malignant transformation is a possible rare and most extraordinary hazard that may occur during continuous cultivation of mammalian cells *It is not known that such* transformation is an inescapable hazard or even that it occurs from other than extrinsic cause Altered cells are a legitimate and fascinating objective study for both virologists and cancer biologists As an approach to this problem a broad program aimed at the derivation of new cell lines and the characterization of the new strains of both normal and malignant cells has been instituted in our laboratories These criteria are shown in Table 109 Elsewhere we emphasized the importance of rigidly standardized technic for propagation of cells used in virus research and described methods for concomitant study of the growth and the metabolism of mammalian cells Such broad characterization of cultivated cells as indicated in this table must be applied in conjunction with search for means of establishing human cells in *continuous culture under known environmental* chemical and physical conditions calculated to preserve normality and not to induce abnormality

The points brought out in this table include *Origin* It is absolutely imperative that the species origin of each cell line employed be established whether derived from man mouse or other animal This is part of the identification

The Growth Phase lag rate duration of unfed cultures These lines vary remarkably

TABLE 109 COMPARATIVE PROPERTIES OF HUMAN CELLS IN CONTINUOUS CULTURE

Origin confirmation as human and not other species

Growth phase lag rate duration in unfed cultures

Size volume and extension

Metabolism a ratio of glycolysis to respiration
b oxygen consumption (Q_{O_2})
c evidence for inefficient or uncoupled respiration
d essential nutritional elements

Transplantability to hamsters and monkeys normal and pretreated with cortisone and x irradiation

Virus spectrum response to viruses of T 1 3 poliomyelitis vaccinia and herpes

Pe nse t irradiation dosage required for
(a) destruction
(b) inhibition of capacity to produce virus
(c) inhibition of capacity to multiply

Size volume and extension

Metabolism a battery of tests of metabolism are applied as routine

Transplantability to hamsters and monkeys normal and pretreated with cortisone and x irradiation A cancer patient is a sick patient

Virus Spectrum response to a battery of viruses and not to a single virus which of itself may well be altered

Response to Irradiation as outlined by Dr Puck in part

More attention must be paid to the potentialities of the normal cell Otherwise we should have to dismiss all cells in culture inclusive of monkey kidney cells since they grow as cells in vivo do not Methods and results discussed by Drs Eagle and Puck can be extended (1) to indicate in terms of plating efficiency the suitability of defined media for optimum propagation of cells direct from normal tissue (2) to *define nutritional requirements of such normal* cells and (3) to supplement defined media so as to permit propagation of particular and the more fastidious cells Finally we need improved methods for differentiation and identification of cell types at both species and cellular levels The characteristics of each cell line must be established Meanwhile to reveal the nature and the mechanism of these intriguing alterations of cells so that the changes can be avoided or produced at will such work as we have heard reported by Dr Parker must continue

As a discussant I believe that we have been most fortunate in having this excellent group of papers presented today

Dr Dulbecco We discuss some of our recent results on the nature of variants of HeLa cells which can be obtained by exposing the cells to poliomyelitis virus These results also have some bearing on the problem of virogeny in *these cells*

The variants were isolated by following the procedure of Ackermann and Kurtz Cells of the S3 strain were infected at a multiplicity less than 1 with an attenuated strain of polio virus Type 3 kindly supplied by A B Sabin The infected cells were then cultivated in a medium containing antiviral serum The progeny of the surviving cells was exposed again to virus in a similar way Five serial steps were carried out all similar except for the multiplicity of the virus which was steadily increased to reach the value of about 8 p f u per cell in the last step

The cell population obtained after this procedure is called the resistant culture It was composed by elongated fusiform cells differin from those of the usual S3 type in agreement with the previous findings of Kurtz

This resistant culture was investigated in several ways A first series of experiments concerned the fate of the culture in subsequent mass transfers At every transfer 2 cultures were set up 1 with and 1 without antiviral serum For 6 serial passages the culture without antiserum degenerated after a few days abundant virus could be found in the supernatant The cultures containing antiserum did not degenerate when their cells were trypsinized and plated on monolayers of monkey kidney cells for viral plaques a proportion of them was found to be virus producing in the early passages This *proportion was 5 per cent* at the second passage and 1/10 000 at the third passage This proportion was not significantly different when determined on monolayers of giant HeLa cells obtained after x ray treatment

At the seventh passage the cells of the culture with antiserum no longer produced plaques on monkey kidney cells the culture without antiserum did not degenerate In subsequent passages without antiserum the progeny of this culture did not degenerate any longer and no production of virus could be detected *These* results show that the cells of the original resistant culture were not permanently virogenic although the cultures kept with antiviral serum displayed what appeared to be a transient virogenicity This apparent virogenicity may well be due to a complex dynamics of cellular infection by virus and of virus inactivation by antiserum and may be therefore of trivial nature The nature of the apparent virogenicity was *studied further by* experiments that gave the results to be described presently

It was found that the fusiform cells present in the resistant culture are not necessarily associated with virogenicity for two reasons The first reason is that nonvirogenic population derived from the seventh passage of the resistant culture had the same fusiform cell type as the apparently virogenic cultures of earlier passages. The second reason is that cells of the fusiform type can be found with a frequency of a few

per cent in the untreated S3 culture as shown by examining unselected clones

To clarify further the nature of the cells present in the resistant culture pure lines were obtained from its seventh avirogenic passage by an improved technic which allows the isolation of cell populations derived with certainty from a single cell This technic is a development of the single-cell technic described by Lwoff *et al*—individual cells obtained by trypsinization were transferred under the microscope to large drops of medium kept under a thick layer of paraffin oil The individual cells started to multiply after a lag period of less than 24 hours and divided regularly with a constant doubling time of 20 hours to give rise to large populations Among the pure clones thus isolated 3 different morphologic types were observed 1 very elongated called F (fusiform) 2 less elongated called I (intermediate) characterized by a typical pattern of the clones and 3 polygonal called P Cells of F and I type were main components of the seventh passage culture cells of P type were rare

The virus resistance of these 3 pure cell lines was investigated by determining the proportion of cells able to produce a clone as a function of the multiplicity of virus infection I and F cells were found to be more resistant than S3 cells whereas the resistance of P cells was almost identical to that of S3 cells The nature of the resistance was brought to light by the survival curves These showed that absolutely resistant cells were absent the increased virus resistance of the F and I line could be attributed to a decreased probability that a cell became infected when a virus particle became attached to its surface Other experiments showed that once the resistant cells became infected they reproduced the virus like the more sensitive cells The varying slope of the two curves shows that both populations contained cells of different resistance although the F population was much more homogeneous than the S3 population Both populations contained a fraction of highly resistant cells (lesser slope) for which the probability of infection was about 1 70 of that of the most sensitive cells contained in the S3 culture The S3 culture contained about 5 per cent of the highly resistant cells the F culture contained about 50 per cent of them in addition the F culture did not contain any important fraction of cells of high sensitivity It is interesting that the proportion of highly resistant cells present in the S3 culture was of the same order as the proportion of the fusiform cells found in noninfected S3 cultures

The phenomenon of temporary virogenicity of the resistant cultures at the early passages was investigated further by studying the fate of individual infected F cells An F culture was infected and the infected cells were trypsinized and transferred individually to drops of fluid under paraffin oil in the presence of antiviral serum The cells were examined daily and the medium was assayed periodically for presence of virus About 200 cells were studied Of these about 75 per cent degenerated within the first 24 hours virus was found in their supernatants in 80 per cent of the cases The rest survived and gave rise to clones the supernatant of which was always free of virus In 3 cases virus was found in a drop containing a cell that had divided however in all 3 cases degenerated cells appeared fairly soon in the drop none of these cells gave rise to a permanent clone

The experiments show that the F cells do not become permanently virogenic with a frequency greater than 1 per cent A temporary carrier state lasting not longer than a few divisions seemed on the contrary to occur in about 1 per cent of the cases

In conclusion the effect of the virus could be shown to consist of a selection for special preexisting cell types These cellular types differ in their virus resistance from the majority cell type present in the population before infection they are different also in other characteristics such as their morphology as has been shown and some physiologic properties which were not discussed They do not appear to be or to become permanently virogenic

BIBLIOGRAPHY

Ackermann W W and Kurtz H J Exper Med 102 555 1955
Lwoff A Dulbecco R Vogt M and Lwoff M Virology 1 128 1955

Dr Melnick My remarks are an extension of Dr Eagle's paper because in New Haven Dr Rappaport in particular has been concerned with the development in a synthetic medium of the growth of monkey kidney cells and also the growth of virus in such cells

TABLE 110 SYNTHETIC MEDIUM (SM 2) FOR GROWTH OF MONKEY KIDNEY CELLS

AMINO ACIDS	MG /LITER	VITAMIN MIXTURE	MG /LITER	BASIC SALT	GM /LITER
1 isoleucine	300	Folic acid	40 0	NaCl	8 0
1 leucine	200	Biotin	0 15	KCl	0 4
1 lysine	60	*Nicotine acid*	0 15	$MgCl_2$ 6H O	0 1
1 histidine HCl	10	Pyridoxine	0 115	$MgSO_4$ 7H O	0 1
1 valine	170	Thiamine	0 15	Na HPO	0 06
1 arginine	60	Pantothenate	0 10	NaH_2PO_4	0 06
1 phenylalanine	90	Riboflavin	0 15	$CaCl_2$	0 4
1 tyrosine	145	Vit B_{12}	0 015	$NaHCO_3$	See note
1 tryptophan	5	Choline	1 00		
1 methionine	12	Inositol	3 50		
1 threonine	60	Ascorbic acid	50 00		
1 cysteine HCl	100				
glycine	150				
1 glutamine	280				

MISCELLANEOUS	MG /LITER	TRACE ELEMENTS	M x 10 ⁶/LITER
Lecithin	25	Fe $(NO_3)_3$ $9H_2O$	2 0
Cholesterol	10	CoCl $6H_2O$	0 ?
Ethyl alcohol (a diluent for lecithin and cholesterol)	590	$MnCl_2$ 4H O	0 5
Glucose	1 000	ZnSO 7H O	3 0
Pyruvate (sodium salt)	500	(NH)$_2$$MoO_4$	1 0
		$CuSO_4$ 5H O	0 4

Notes on preparation

Amino Acids—held as 20 x concentrated combined stock without tyrosine cysteine glutamine Tyrosine held as 0 2 per cent stock cysteine held as 10 per cent stock at 4 C glutamine held as 2 per cent stock at −20 C

Amino acids sterilized by filtration

Vitamin Mixture—held as 100 x conc stock without ascorbic acid Sterilized by filtration

Miscellaneous—Lecithin (Pfanstiehl ex ova) held as 10 per cent stock in absolute ethanol cholesterol as 2 per cent stock in absolute ethyl alcohol

Basic Salt—held as Hanks s salt 10 x conc with glucose supplemented with 0 26 Gm /liter of CaCl and with bicarbonate after the medium has been made up $CaCl_2$ held as 10 per cent stock NaHCO concentration for outgrowth from trypsinized suspensions 1 1 Gm /liter for replenishment 1 9 Gm /liter

Trace Elements—held as separate stocks 0 05 per cent conc Fe (NO) sterilized by filtration

To prepare 1 liter—add in order to 750 ml distilled demineralized H O

Hanks s 10 x conc with glucose		100
SM 2 Amino acid conc 20 x		50
Tyrosine 0 2 per cent heated to dissolve		70
Glutamine 2 per cent		14
Cysteine 10 per cent		1
Vitamin mixture 100 x conc		10
Ascorbic acid	10%	0 5
Lecithin		0 25
Cholesterol		0 5
$NaHCO_3$ (7 5% solution)		15–25 (see note above)
$CaCl_2$	10%	2 6
$ZnSO_4$ 7H O	05%	0 2
Fe (NO) $9H_2O$	04%	0 2
CuSO 5H O	05%	0 05
CoCl $6H_2O$	01%	0 05
$MnCl_2$ 4H O	01%	0 10
(NH)$_2$MoO	05%	0 2

The synthetic medium which Dr Rappaport has developed is shown in Table 110 and as you can see contains the essential amino acids and also cystine glutamine and another require ment which Dr Rappaport has found to be essential for monkey kidney cells namely gly cine The notable difference between this me dium and those which others have used has een the fact that there is additional calcium in this medium and trace elements but particu larly the amounts of calcium used are about 3 to 4 times that commonly used in salt solutions such as Hanks s or Earle s This particular me dium will support the exponential growth of monkey kidney cells for a few weeks with a mean generation time of 24 hours This curve (Fig 169) was constructed by determining the cell count daily on a number of cultures by a method that Dr Rappaport has recently devel oped of measuring decoloration of phenol red 15 seconds after it is added to such cultures It should be emphasized that the addition of serum to this synthetic medium does not increase either the rate or the duration of cell growth

Two requirements of the physical environ ment have been established As I mentioned a concentration of calcium 3 to 4 times higher than that in Hanks s original formula is neces sary for growth Without this calcium supple ment the cells stop spreading along the glass the edges of the cells become thickened and the cells granulated

Even more critical than the presence of glycine or the concentration of calcium is the type and the conditioning of the glass used for the cul tures The medium SM 2 supports exponential growth on soft glass but will not support even survival for 3 days on hiird pyrex glass unless the glass is conditioned by a preliminary rinse with sodium hydroxide This is in order to repla e the surface bound proton with sodium ion

It has been found that polio virus is propa gated in SM 2 monolayer cultures in a salt glucose medium in the ab ence of any exogenous nitrogen source as well as in the complete me dium containing calf serum lactalbumin hy drolysate The rate and the amount of virus produ ed during the fir t 24 hours after the cells are transferred to the salt solution is independent of any exogenous carbon sour e

Thus the nitrogen the organic carbon and

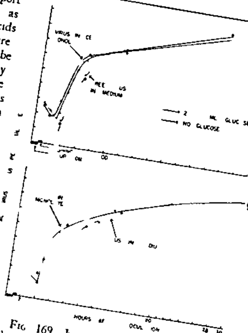


Fig 169 Kinetics of polio virus syn thesis in monkey kidney cells in the pres ence and the absence of glucose Nine-day old monolayer cultures of monkey kidney cells washed and then inoculated with 3 x 10³ plaque forming units per culture Virus suspended in Hanks s salt solution with or without glucose After 1 hour cultures washed free of unadsorbed virus and replenished with Hanks s salt solu tion Intracellular (plus cell bound) virus and free virus in the medium titrated by plaque technic at times indicated

all the energy for the synthesis of virus must be derived from the endogenous reserves of the cell Since the total endogenous polysaccharide of the culture of 1 000 000 monkey kidney cells varies between 30 and 50 gamma equivalents of gluco e and since the cultures can utilize 00 gamma of glucose per hour it would seem that the amount of energy used for synthesis of virus during the 24 hours is a very small fraction of the cell s total energy producing capa ity

Only two constituents of SM 2 have been found to affect the course of virus propagation Typical data for the effect of cystine in this system are given in Tal le 111 At any given glucose concentration cystine decreases the time when cytopathogenic changes are complete and

TABLE 111 STIMULATORY EFFECT OF CYSTEINE ON VIRUS SYNTHESIS IN M CULTURES IN SALT GLUCOSE SOLUTION

MEDIUM HANKS S SALT SOLUT ON SUPPLEMENTED WITH		TIME OF HARVESTING (WHEN CPE IS COMPLETE)	PFU PER CULTURE	VIRUS YIELD PER CELL
CARBON SOURCE GLUCOSE (MG /ML)	NITROGEN SOURCE CYSTE NE (μG /ML)	(HRS)	(LOG)	(PFU)
3 0		90	8 8	60
	50	60	9 4	1 0:0
0 9	—	49	9 0	400
	50	43	9 4	1 050
0 3	—	49	8 6	0
	50	49	9 0	400
3 0		95	8 4	110
	50	55	9 0	400
0 9	—	71	8 7	00
	50	55	9 2	630
0 3		71	8 3	7
	50	47	9 1	5 0

M u tu e n bo le ea h nocu a ed h an a e a e of 5 o 10 PFU su pended n H nk solu n Af 1 hr 3 C cu u es e e hed 3 tim h PBS and p n hed h 10 ml of H nk s sa so uti pp m nted as nd a d



FIG 170 Plaque inh b t on by presence of glycine n o erlay at mola ty nd a ed C — control culture n absence of glyc ne

increases the yield of plaque forming units per cell If the yields obtained in the presence of cystine are corrected for thermo inactivation of virus it is seen that monkey kidney cells can produce at least 2 000 active virus particles per cell

As already mentioned glycine is required for the growth of monkey kidney cells However glycine inhibits the synthesis of polio virus Figure 170 shows that in the presence of glycine plaque numbers under a protein free synthetic medium used in the agar overlay are reduced and the rate of growth of the plaques is re tarded Varying concentrations are shown in the bottles the control is on the left and the falling concentrations go from left to right

The results in Figure 171 show that there is a 2 to 3 log inhibition of polio virus synthesis during the first 40 hours in the medium con taining glycine A replenishment of this me dium and thus of the glycine every 10 hours prolongs the inhibition of virus growth

It appears from these studies then that the synthesis of polio virus by monkey kidney cells is not an invariant consequence of the absorp tion of the virus to the cell but actually may be ontrolled by specific transients in metabolism These transients (ordinary amino acids like cystine and glycine) may be favorable or un favorable to virus synthesis and they may be induced by compounds which are physiologi for the cell

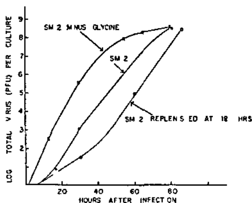


Fig 171 Effect of glycine on propaga tion of Type 1 polio virus using complete growth medium SM 2 and SM 2 minus glycine Ten-day-old 2-oz bottle cultures grown in SM 2 were inoculated with 5 10 PFU After 1 hour at 37 C cultures were washed and replenished with indi cated medium There was a 2 to 3 log inhibition of polio virus synthesis during the first 40 hours in the medium contain ing gly ine

BIBLIOGRAPHY

Rappaport C and Melnick J L Fed Proc 16 429 1957

Dr Lwoff All those who have had animal cell cultures for some time have noticed morpho logi or physiologic changes in them There fore it was important to find out whether these changes come from a selection of a cellular type pre-existent in the culture or from a spontaneous or indu ted change

In order to solve that problem it was impor tant to develop a technic whi h would facilitate the isolation of clones in other words popula tions emanating from a single cell The first clones were obtained by the Wilton Earle group The techni was extremely elegant but some what lengthy and delicate Thanks to the me dium defined by Theodore Puck it is possible today to isolate clones with the greatest of ease According to Puck one can obtain clones by fertilizing isolated cells in petri plates and by isolating colonies developed or emanating from a single cell

Also it is possible to follow a safer technic developed by Raymond Parker of taking a single cell with a micropipette and injecting it into a droplet of a medium under a paraffin slide You then can take the clone and fertilize it in a bottle There is also a simpler technic—one used in the Pasteur Institute—that of isolat ing a single cell with a micropipette and in jecting it directly into a tube

Therefore it is possible nowadays to obtain clones thanks to a great variety of technics That is why physiologists and geneticists can tackle a number of basic problems affecting animal cells One of these is the problem of the origin or the nature of the cell variations which have been observed in vitro

These are not necessarily—as was shown by Raymond Parker—the product of a selection of variations existing in the cells Rather these variants are the result of the change affecting a single cell—a change a modification whi h can

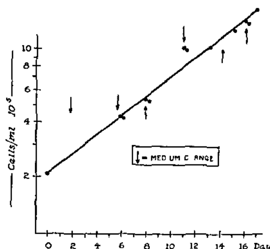


Fig 172 Growth of L cell in spinner culture

take place a few days after this in vitro culture

The monkey kidney cell then is linked to *the number of chromosomes* *What is note*worthy is that such modifications seem to take pla e under the influence of the change of the medium This is according to Raymond Parker s hypothesis of inducted multiplication coming from the medium—medium induced changes The understanding of this is particularly impor tant for virologists because we did know as a matter of fact that the variants of the cells which we have gotten in vitro can go from sensitivity to the virus to sheer resistance and we also have found that such modifications can bring about changes in the sensitivity to diph theric toxin The in vitro cell studies then will *shed a great deal of light on a number of* points which are still much in the dark as far as cell pathology is concerned

Therefore I would refer to a single point raised by Harry Eagle He said that the mam mal needs 8 amino acids and that the mammal cells need 13 of them as they are studied in vitro We know that for the syntheses of the amino acids a number of enzymes are necessary The synthesis of a given enzyme is governed by a single gene and depends on the specific indu tor This specific inductor is exogenous for the nonconstitutive enzymes but be they adaptive or constitutive enzymes for synthesis a specific inductor is needed in either case

A micro-organism as a whole is a well organized totality The mammal cell is an inte gral part of a general whole and we know that the functioning of a mammal cell will depend on the other cells on substances such as hor mones which are produced by the other cells of the organism What is true of the hormones could well be true for these inductors For example during the development of the cell differentiation could well be linked to modifica tion of the synthesis potency of the specifi in ductors The liver cells for example could yield a specific inductor for the syntheses of given enzymes which would act as an inductor for the kidney cells *In vivo kidney cells would* receive this inductor through the blood whereas the kidney cell preserved in vitro would be without this inductor and therefore would not synthesize a specific enzyme This is only a hypothesis but the fact that we can talk about such a premise and the fact that its importan e is worthy of or warrants a study shows that the culture of tissues up to now enables us to *tackle a number of fundamental* basic problems in the field of physiology and cell biology

The results which have been obtained by Harry Eagle by Puck by Parker by Alice Moore which have been presented us this morn ing are particularly important

Dr McLimans The important papers p sented have been based primarily on the use of glass monolayer tissue cultures a technic that appears to be ideally suited for certain types of investigations but is not without lim at ns in some other aspects particularly in securing suffi cient quantities of cells for the production of *viral antigens for* biochemical studies or physio logic investigations

The submerged culture technic was origi nally introdu ed through the imaginative efforts of our moderator Dr Wilton Earle utilizing a shake flask system *I sincerely hope* that he will be able to comment on some of his own observations in this field

Investigations conducted in our laboratories through the collaborative efforts of my col leagues Drs Davis Ziegler *Thomas* Kucera and Larson have established the feasibility of obtaining continuous logarithmic growth of stable mammalian tissue cell l nes (Fig 172) as discrete units (Fig 173) in agitated fluid sus pension referred to by us as *submerged culture* in volumes of 25 ml to 5 gallons and cell con

centrations of 1 to 5 million cells per ml for continuous periods of time up to 5 months Additionally it has been demonstrated that polio virus proliferates in submerged culture cells (strain HeLa) to high titer exhibiting a more rapid rate of absorption and release of the virus with earlier appearance of cytopathologic changes than in the instance of the same host cell grown as a monolayer on glass

The use of the submerged culture process for the production of viral antigens certainly possesses great advantages over conventional techniques However since a stable host cell line of human or monkey origin must be used for propagation of polio virus and since an adequate criterion of malignancy for mammalian cells has not been established the fear of transmission of a malignant inducing principle precludes the use of such a system for the production of agents designed for parenteral use in man A proposed solution to this problem in the case of polio virus would be the establishment in submerged culture of a stable cell line sufficiently far removed from man specieswise as to obliterate the fear of use of such a viral vaccine on the grounds of associated stigma of malignancy

The tremendously important discovery by Westbrook Sheffield Smith *et al* that a stable rabbit kidney epithelial line (called ERK or subline KD) would support the propagation of all 3 types of polio virus prompted us to investigate this cell line in submerged culture We have confirmed their observations in that in monolayer cultures polio virus Types 1 2 and 3 produce typical cytopathology although to low titer on the first passage in the KD line On serial passage of the virus strains adaptation took place to the extent of 10^{4} to $10^{1.5}$ titers Submerged culture studies were then promptly initiated with this cell line and the following results achieved

Sustained logarithmic growth of the KD cell as discrete units in a spinner culture (100 ml volume) (Fig 174) in the New Brunswick Fermentor (3 liter volume) (Fig 175) and in the stainless steel water jacketed impeller agitated fermentor (5 gallon volume) (Figs 176 and 1) was readily achieved employing Eagle s medium with the daily addition of arginine and inositol It should be noted that on the basis of past experience in the antibiotic

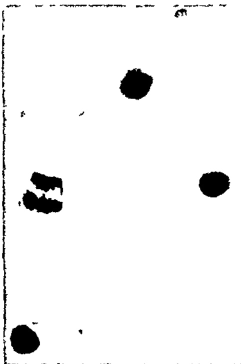


Fig 173 L cell (Earle) grown in submerged culture Delamater s stain (× 850)

industry one could predict from the results obtained in a 5 gallon fermentor the success of a venture in a much larger unit

Growth response curves for the propagation of Type 3 polio virus in serum free Eagle s medium using 4 indicator cell systems—the KD cell the HeLa the monkey testicular cell line and the monkey kidney line—were obtained from the 5 gallon fermentor as shown in Table 11? Initial viral inoculum was adjusted to approximately 1 tissue-culture infectious dose per 2 to 4 000 cells Infectious titers as illustrated of $10^{?}$ and 10^{-5} or better at some points were obtained in less than 40 hours The host cell response to infection was characterized by a rapid drop in viability—viability as loosely judged by staining with trypan blue—but not a drop in total cell count and from a cytologic viewpoint the formation of a perinuclear mass indistinguishable from that observed with HeLa

Since the frequent medium changes—?-day

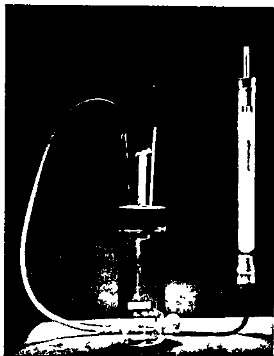


Fig 174 The spinner vessel

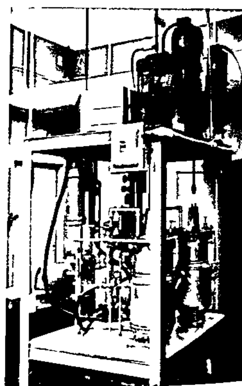


Fig 176 The 20-liter fermentor

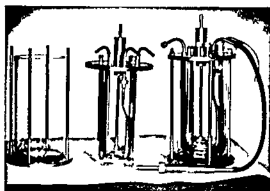


Fig 175 New Brunswick fermentor (N B F) Assembly

to 3-day intervals—become laborious and impractical from the viewpoint of serum requirements particularly with large volumes a search was initiated to find either a substitute for serum or a method of circumventing the necessity for making frequent medium changes It has been observed in our laboratories that the daily or bidaily addition of arginine permits the maintenance of cell growth in fluid suspension without medium changes for the period of 10 to 30

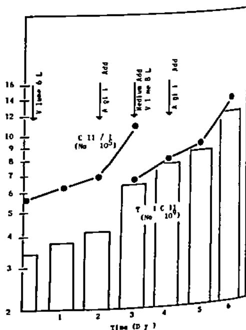


Fig 177 The growth of strain KD cells in the 20 liter fermentor

TABLE 112 HOST CELL (STRAIN ERK/KD)/POLIO VIRUS (T 3_{KD8}) INTERACTION IN 20 LITER FERMENTOR

TIME (HRS)	CELLS PER ML × 10⁴ CONTROL	INFECTED	VIABILITY (%) CONTROL	INFECTED	TCID₆₀/ML M K	M T
0	45	45	82	82	2 5	3 0
3	47	44	73	78	3 0	2 8
6	47	44	78	81	4 5	5 0
12	43	52	87	80	8 0	6 0
21	50	48	80	80	8 0	5 8
24	52	46	80	40	7 0	6 8
27	61	59	79	31	7 5	6 8
33	67	61	78	15	6 0	7 5
39	65	65	81	10	6 5	7 5

1 TCID₆₀ inoculum per 2 880 cells (KD tetr) per 1 406 cells (MK t t)
NOTE Medium supplemented with sera from Eagle ... supplemented with doubled amounts of glutamine and arginine

days Figure 178 demonstrates the role of arginine at various levels in maintaining cell growth as expressed in terms of per cent over the control

In summary the poliomyelitis vaccine as currently produced utilizing primary monkey kidney cell cultures propagated as monolayers on glass surfaces is costly creates problems relating to contaminants raises questions as to the maximum antigenic mass that could optimally be achieved and is to be sure subject to the ready availability of a constant supply of monkeys It is hoped that eventually viral antigens will be produced in such a manner as to provide the maximum number of immunizing doses at the minimum cost for all men In short can we take the monkey business out of the polio vaccine production?

DR CHANG I congratulate the speakers of this morning on their fine presentations on cell nutrition acquired changes and genetics I do not feel qualified to make comments on all these interesting subjects therefore I confine my discussion to Dr Eagle s paper on cell nutrition

We have approached the problem of the nutritional requirements of human cells with methods similar to those of Dr Eagle We use only the HeLa cell and the conjunctival cell derived from malignant and normal human tissues respectively While many of our results are in agreement with Dr Eagle s there also are major areas of disagreement I would like to present briefly the disagreements to illustrate a few interesting points

On the salt requirements of the HeLa cell Dr Eagle showed that potassium sodium magnesium calcium and phosphate were and that bicarbonate was not essential for cell multiplication and survival Through the use of a carbon dioxide trapping device Dr Geyer and I showed that bicarbonate was also an essential for cell propagation We have found that the ordinary medium without the addition of bicarbonate contained about half a millimol presumably through absorption from the air Through the use of this alkaline trapping device the bicar

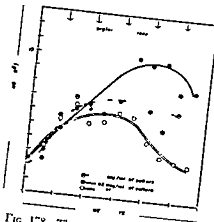


FIG 178 The effect of arginine on the proliferation of L cells in submerged culture

bonate concentration was reduced to below the limits of the method of Van Slyke and Neill While it is true that the HeLa cell would multiply without the addition of bicarbonate they invariably undergo slow degeneration when the absorbed bicarbonate has been removed I think this illustrates an important point through the use of a more refined technic another ion has been shown to be essential

The necessity of incorporating whole or dialyzed serum into chemically defined mediums in order to obtain satisfactory growth poses a very great stumbling block in the study of the nutrition of the human cells Dialyzed serum is a mixture of many many components one or more of which are essential for cell growth Different sera are known to differ in their growth sustaining properties and I have presented this in a previous conference I would add one more important difference between the sera—their enzyme activities We have found that cell multiplication would occur with sucrose as the sole source of carbohydrate in a dialyzed horse serum but not in a dialyzed human serum Subsequently it was shown that the horse serum but not the human serum possesses the ability of hydrolyzing sucrose into glucose and fructose The implication is clear the results obtained in a particular study may be influenced greatly by the source of the serum used

I would bring up once again my inability to grow HeLa cells in dialyzed serum diluted in Eagle s basal medium The ability to propagate HeLa cells in this medium is the whole basis of Dr Eagle s depletion and repletion experiments In a more recent publication Dr Eagle reported that he also was unable to grow HeLa cells in dialyzed serum diluted in Eagle s basal medium as originally described and that inositol is an additional growth factor He attributed such drastic difference between our results and his results as well as the difference between his earlier and more recent results to the completeness of dialysis I find it difficult to accept this explanation By dialyzing 10 cc of serum against 2 changes of 200 cc of saline for only 2 hours the serum no longer would sustain the propagation of the HeLa cells when diluted in Eagle s basal medium as originally described without the inositol So a dialysis of 2 hours will remove the inositol from the serum I make this point for only one purpose—to stress an important weakness of our present cell system How may one be certain that a cell strain 1 year ago and the same cell strain at present still possesses exactly the same properties? If one does find a distinct change in the nutritional requirements how may one prove conclusively that mutation or adaptation has occurred or that a technical error has been committed? Without a method for the reversible arrest of cell metabolism for an indefinite period of time the systematic study of such a problem is extremely difficult

Until the time when a synthetic medium capable of supporting growth and a method for the reversible arrest of the metabolism of these human cells become available one should be more cautious in drawing conclusions from exp rimental data ob ained by the methods currently used

Dr Coriell I agree with all the speakers on the panel that when cell lines derived from normal tissue adapt to tissue culture and grow vigorously interesting alterations in morphology metabolism and reactivity frequently develop It has been suggested but has not yet been established that these changes are synonymous with becoming malignant

The criterion of transplantability to cortisone and x ray treated rats or volunteer cancer patients as described by Dr Moore should be a reliable test for malignancy but these data also may lead to erroneous conclusions as we have tried to point out by injecting *normal* monkey kidney cells subcutaneously into treated rats and following the course of the lesion by serial biopsies In brief the normal cells grow during the first week producing nodules composed almost entirely of epithelial cells which become surrounded by fibroblastic cells and disappear during the third of fourth week

In our hands the human conjunctiva and kidney lines (Chang) behave like normal monkey kidney whereas HeLa cell nodules persist longer and differ in many microscopic details.

The practical point is that if one examines between 7 and 14 days the nodules from rats injected subcutaneously with normal *or* malignant cells the microscopic appearance may have similarities which can be misleading Normal cells have hidden potentialities when placed in the treated animal as Toolan has previously

pointed out The quantity of cells injected is one important factor but we must realize that in common with all protoplasm even normal cells have a capacity for survival and adaptation to changes in the environment There is not time here to enumerate the known mechanisms by which this may be accomplished We have now injected over 1,200 cortisone and x ray treated rats *intraperitoneally* with cells from normal monkey kidney 4 malignant cell lines and 3 long term lines derived from normal human tissue By this route the distinction between cell lines is very sharp that is malignant cells grow progressively and kill the majority of the rats while the normal cells do not grow at all

However results with another cell line derived from normal tissue are of considerable interest When we injected monkeys subcutaneously with the cynomolgus heart cell line (SCH) adapted to tissue culture from normal monkey heart by Salk and Ward a cellular subcutaneous tumor developed which enlarged for 1 to 2 or 3 weeks and then regressed Great variation was noted in individual monkeys Pretreatment of the monkeys with cortisone and x ray did not change the response Following intraperitoneal injection of these cells no tumors were found in monkeys In marked contrast this cell line caused massive fatal tumors when injected intraperitoneally into treated rats SCH cells were even more invasive than HeLa cells and produced nonfatal tumors in untreated rats These rats were subsequently immune to rechallenge even though pretreated with cortisone and x ray at the time of rechallenge

Chromosome analysis of this cell line (SCH) shows that it is heteroploid and quite different from normal monkey kidney Heteroploidy is not synonymous with malignant change as shown by chromosome counts on Chang conjunctiva Chang kidney monkey kidney HeLa and SCH cells

By precipitation and complement fixation tests the SCH cell is closely related to human cell lines (HeLa conjunctiva and kidney [Chang]) and intestine (Henle) and gives little cross reaction with fresh monkey kidney monkey heart monkey serum or fresh human heart or human serum This suggests that all long-term established cell lines acquire common antigens not present in the original normal cell

I re-emphasize that temporary survival or growth of transplanted cells in a foreign host or treated animal does not indicate that the cell is malignant However if the cell grows progressively and kills a cortisone and x ray treated rat it is certainly malignant for that animal The true significance of these observations is not clear and much more study is needed While exploring the meaning of various other methods and technics of characterizing cell lines I suggest that a cell line may be classified tentatively as malignant if it produces progressive fatal tumors in the treated weanling rat injected by the intraperitoneal route

BIBLIOGRAPHY

Corell L L McAllister R M Greene A Flagg W Tall M and Wagner B M In vitro and in vivo studies on a tissue culture cell line derived from normal monkey heart J Immunol 80 147 1958

Corell L McAllister R and Wagner B Criteria for determining malignancy in tissue culture cell lines in the albino rat in Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) p 341 New York New York Acad Sc 1957

Foley G E and Handler A H Differentiation of normal and neoplastic cells maintained in tissue culture by implantation into normal hamsters Proc Soc Exper Biol & Med 94 661 1957

Salk J E Poliomyelitis vaccination in the fall of 1956 Am J Pub Health 47 1 1957

Toolan H The potentialities of normal cells implanted in cortisonized and/or x radiated hosts Cancer Res 17 248 1957

Dr Salk I recall some 6 years ago the International Conference in Copenhagen when John Enders presented the results of his magnificent studies with tissue culture which suddenly blossomed to an enormous activity when touched with polio virus Dr Paul predicted the end of the monkey era

Today's discussants and many of the rest of us feel strongly that the end of the monkey era will come when the monkey can be replaced completely in the preparation of polio virus vaccine and other vaccines as well The feeling was well expressed by Dr Syverton when he said that the specter of malignancy hovers over the question as to whether or not one can use an independently propagating cell or self propa

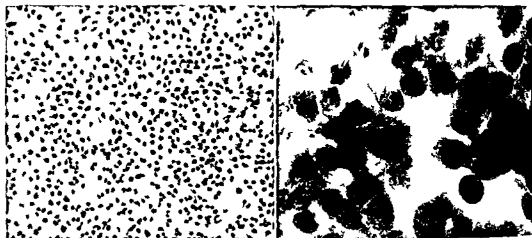


Fig 179 (*Left*) Photomicrograph of a culture of the cynomolgus heart cell (*Right*) Higher magnification of the same cell culture

gating cell for the preparation of such material He urged further that investigations be done rather than resorting to hypotheses and speculation Dr Cornell commenting on Dr Moore's excellent studies urged some equivocation as to the definition of malignancy The question then arises Is this Conference going to end without some firm and definitive idea that before another Conference or before another year goes out some definite steps will be taken to supplant the monkey kidney in the preparation of polio virus vaccine and thus to eliminate one of the tremendous problems confronting those interested in vaccine technology? Dr McLimans presented some excellent studies Perhaps he and the British workers who introduced the rabbit cell may well have the answer to the question

In the meantime quite by chance we encountered the cynomolgus heart cell which Miss Ward observed in the course of cultures being investigated for quite another purpose. When we found some of the properties to which Dr Cornell has already referred and I shall not repeat we looked into this question—the question of this cell and its usefulness from the viewpoint of malignancy Here was a cell that could be tested in the host from which it was



Fig 180 (*Left*) Photograph of the 48th subculture (*Right*) Higher magnification of cells from the 48th transfer





Fig 181 (*Top left*) Photograph of monkey that was inoculated with 1 000 000 cells subcutaneously (*Top right*) Stripping of the capsule of the large tumor in the same monkey (*Bottom*) Cross section of tumor



Fig 182 (*Left*) Photomicrograph of a biopsy of the tumor shown in Figure 181 (*Right*) Higher magnification of the same tumor

derived a test not so easy to carry out with the HeLa cell or other cells of human origin I will show the results of our efforts in this direction Figure 1 9 left shows a photomicrograph of a culture of the cynomolgus heart cell You can see the active division that is going on mitotic figures are abundant This is one of the most actively growing cells that those who have compared cell growth have seen Figure 1 9 right is a higher magnification of

the same cell culture This was the 27th transfer The cell has gone through some 60 or 70 transfers now Figure 180 left is a photograph of the 48th subculture and you will see the similarity in morphology between this and the first Figure 180 right is a higher magnification of cells from the 48th transfer

Figure 181 top left is a photograph of a monkey that was inoculated with 1 000 000 cells subcutaneously some 6 weeks earlier The lower site was inoculated with ¼ that amount—a million cells a quarter of a million cells and a quarter of that in the lowest area This was done in titration You see the large tumor that developed at the upper site when 1 000 000 cells were inoculated This was the first time that such an observation had been made All other times that monkeys had been inoculated we had observed the kinds of tumors that Dr Coriell referred to that lasted a matter of 2 weeks and then disappeared The large tumor was excised Figure 181 top right shows the stripping of the capsule it was fairly free and not adherent Figure 181 bottom is a cross section of the tumor showing its approximate size The surgeon who removed it said that to him it would have looked like a benign tumor if he had removed it from a breast Figure 182 left is a photomicrograph of a biopsy of the tumor this shows the fibrous part of the fibrous capsule and no evidence of invasion Figure 182 right is a higher power magnification of this same tumor from this tumor cells were grown

Figure 183 left is another monkey inoculated at the same time showing smaller tumors These disappeared ultimately Other animals in the same group did not develop any tumors Where 6 sites were inoculated (Fig 183 *right*)

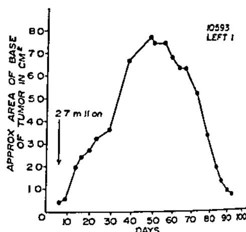


Fig 184 Growth and regression of subcutaneous tumor in Rhesus monkey induced by cultured monkey heart cell

and in this instance I believe that these animals received about 2 000 000 to 3 000 000 cells in each site tumors of this kind appeared in about 1 out of 20 monkeys so treated. Figure 184 describes the rate of increase and disappearance of tumors when they get to be the size that you just saw In 40 to 50 days they reached their peak and then disappeared by 90 to 100 days—in about 3 months

The purpose of this presentation was to show some of the positive evidences that we have of tumor formation following inoculation of monkeys with this particular cell line We have inoculated hundreds of animals and in no instances have we had either a reappearance of the tumor or anything resembling malignancy Therefore the question arises as to what criteria are needed to make it possible for us to take the next step in preparing polio virus vaccines from continuous cell lines



Fig 183 (*Left*) Photograph of a monkey showing smaller tumors (*Right*) Photograph of another monkey showing tumors which developed at 6 sites of inoculation

General Considerations of Viruses

WEDNESDAY AFTERNOON, JULY 10 1957

Chairman

DR F C BAWDEN
Rothamsted Experimental Station
Harpenden England

Speakers

PROF DR G SCHRAMM
Max Planck Institut fur Virusforschung
Tubingen Germany

DR CARLTON E SCHWERDT
Department of Microbiology
Stanford University
Stanford California

DR ROBLEY C WILLIAMS
Virus Research Unit
Molteno Institute
Cambridge England

DR COUNCILMAN MORGAN
Columbia University
College of Physicians and Surgeons
New York

Discussants

Moderator DR WENDELL M STANLEY
University of California
Virus Laboratory
Berkeley

DR H L FRAENKEL CONRAT
University of California
Virus Laboratory
Berkeley

DR LARS KJELLEN
Virus Department
Central Bacteriological Laboratory
Stockholm

DR CARL F T MATTERN
National Institutes of Health
Bethesda Maryland

DR JOSEPH L MELNICK
College of Medicine
Baylor University
Houston

Structural and Functional Parts of Tobacco Mosaic Virus

PROF DR G SCHRAMM

A virus could be defined as a particle containing ribonucleic acid able to invade a host cell and to multiply inside the cell The question may be raised as to how these biologic functions can be correlated with definite structures of the virus In bacteriophages and several animal viruses are components which enable these particles to penetrate the cell membrane They contain substances reacting with the specific receptors in the surface of the cell and enzymes which destroy the receptors The simple plant viruses apparently do not contain such components They invade the cell only if the membrane is previously damaged mechanically either artificially by rubbing or naturally by insect vectors However we may expect that the external structure of these viruses facilitates the penetration and enhances the stability inside and outside the cell

The nucleic acid plays the dominant part in the multiplication of viruses as conclusively demonstrated by the work of Hershey and Chase with bacteriophages It was demonstrated that only the desoxyribonucleic acid (DNA) localized inside the head of the phage is responsible for the multiplication and furnishes the genetic information The external coat is necessary for adsorption and invasion

In many other viruses too the inner parts consist of nucleic acid or at least of substances with a high content of nucleic acid whereas the *shell is essentially composed of proteins* This was demonstrated for instance for the vaccinia virus for the influenza virus for the virus of fowl plague and for the turnip yellow mosaic virus The same principle is valid for the structure of tobacco mosaic virus (TMV) Our knowledge about the nucleic acid and protein component of the TMV is particularly developed and will be discussed in this paper

STRUCTURE OF TMV PROTEIN

The virus is a rodlike particle with a length of 3 000 Å and a molecular weight of about 40×10^6 It contains about 6 per cent ribonucleic acid (RNA) and 94 per cent protein The first structural concepts of the protein part were obtained by degradation in weak alkaline solution at pH 10 The kinetics of the degradation is rather complicated and not all the details are clear The final products are RNA nucleoproteins and a protein component free of RNA called A protein A protein is homogeneous with respect to sedimentation and electrophoretic mobility At pH 10 the molecular weight is about 90 000 Chemical investigations revealed that A protein is not the smallest subunit of the virus protein By determination of the terminal groups of the peptide chains it was found that A protein consists of 6 peptide units Therefore the total protein of the TMV contains about 2 300 peptide units of a molecular weight of about 17 000 These peptides have the same terminal sequences of amino acids The sequence of the other amino *acids is being* investigated in several laboratories and it is possible that all 2 300 chains are identical

It is interesting that A protein can be reaggregated in acid solutions (pH lower than 6) to rodlike particles resembling the original virus As the electron micrographs show the diameter is the same as that of the virus but the lengths are variable and depend on the pH of the medium Recently Kramer and Wittmann observed that by reaggregation of the A protein which has a lower anodic electrophoretic mobility than *the virus* the electrophoretic mobility changes to that of the original virus The internal structure of the reaggregated A protein was investigated by x ray methods The general features of the reaggregated protein and of the virus containing RNA are the same In both cases the peptide units are arranged in a helix The reaggregated A protein does not have infectivity due to the lack of RNA

In the process of reaggregation several distinct steps are observed The sizes and the shapes of these intermediates and of the final rods can be explained by the properties of the peptide subunits of 17 000 This example makes clear that

even complicated biologic structures are defined by the properties of very small subunits Theoretically 2 300 subunits can give an enormous number of different recombinations In fact only one of these is found in vitro

ARRANGEMENT OF THE RNA IN TMV

From the alkaline degradation some evidence was obtained about the arrangement of the RNA in the virus By short treatment at pH 10 3 A protein and free RNA were obtained and also a nucleoprotein with a molecular weight intermediate between TMV and A protein The nucleic acid content of these particles was higher than in the virus It was assumed that these particles had lost a part of their protein This assumption could be confirmed by electron micrographs In this fraction we observed gaps in the rodlike particles and in these gaps a central strand of RNA was visible We concluded that the TMV consists of a core of RNA that is embedded in a protein shell like the wick in the wax of the candle This conclusion was confirmed and elaborated in more details by Hart using a different method of degradation and particularly by the x ray work of Franklin

FUNCTION OF THE RNA

The virus particles deprived of part of their protein shell had a high infectivity Therefore at least a part of the protein is apparently dispensable for the process of multiplication These results lead to the question whether the infectivity of the nucleic acid could be preserved after complete removal of the protein Because of the instability of the RNA in alkaline solution the preparation of an active RNA by alkaline degradation was not promising A more suitable method is the extraction of the protein by phenol in a neutral medium If a solution of TMV is shaken with phenol in the cold the protein is extracted by the phenol phase After 3 extractions protein is not detectable by chemical means in the RNA fraction Thus the amount must be less than 0 4 per cent of the RNA content Complement fixation showed that the RNA contains less than 0 02 per cent of the native TMV protein Immediately after such a preparation the RNA is infectious The average infectivity is about 5 per cent of that of the same weight of the native virus A comparison of this value with the possible protein content indicates that the infectivity is not due to contamination with intact TMV or residual parts of the protein shell Besides the protein determination two other experiments show that the infectivity is not dependent on residual parts of the protein shell The infectivity is not significantly reduced by repeating the extraction process with phenol and the infectivity of the RNA preparation is not inactivated by TMV antiserum whereas TMV is inactivated under the conditions applied Other control experiments confirmed that the infectivity is not due to intact virus particles or larger nucleoproteins The infectivity is destroyed by ribonuclease which does not attack the intact virus the infective particles have a much lower sedimentation constant than TMV and the stability of the infective particles is much less than that of TMV Thus we are led to the conclusion that the nucleic acid itself is responsible for the infection If protein were involved in the process of infection the amount could only be very small and it would not be A protein

Independently Fraenkel Conrat found that the RNA is infectious after removal of the protein By treatment of the virus with dodecylsulfate he obtained a preparation with a low infectivity This was destroyed by treatment with ribonuclease and by standing at room temperature Therefore the infectivity is due to the RNA itself In the meantime the infectivity of the RNA was confirmed by other laboratories

The difference between the infectivity of the RNA and the virus is considerable If we assume that this difference is caused by the function of the protein during the invasion period it should be possible to increase the infectivity of the RNA by recombination with A protein Recombination of A protein and RNA was tried many years ago in our laboratory in general the results were negative By Fraenkel Conrat and Williams and by Commoner and co-workers an increase of infectivity by recombination was reported Repeating these experiments we found it difficult to reproduce the reported results Further investigations are necessary to elucidate the role of the protein part in the process of infection

Studies have been made to correlate the structure of the RNA with its biologic activity These experiments are difficult because of the instability of the RNA Frequently a decrease of infectivity was observed after 1 to hour at room

temperature The reason for this instability is not clear but the manner of preparation of the RNA seems to be of importance All experiments must be carried out at low temp rature and within a short time The RNA prepared by the phenol method is not homogeneous in the ultracentrifuge The mean molecular weight can be calculated from s_0 and [n] as 1 6 x 10 In the sedimentation diagram a sharp boundary can be observed with $s_0 = 24$ S (c = 0 1 %) followed by a diffuse gradient of material with a lower sedimentation constant The molecular weight of the material in the sharp boundary can be estimated to be about 2 x 10^6 The molecular weight of the RNA core can be deduced from analytic data to 2 x 10^6 The bulk of the infectious material seems to be the undegraded RNA core By differential ultracentrifugation Gierer demonstrated that in the RNA prepared by the phenol method only the fast moving component with a molecular weight of about 2 x 10^6 is infectious From his studies some results were obtained about the structure of the RNA in solution The RNA molecule seems to be a flexible filament with properties intermediate between a rigid rod and a random coil The high molecular RNA has a lower extinction at 260 millimicrons and a much higher optical rotation than its nucleotides Probably the nucleotides are arranged in a definite order in the direction of the fiber

Gierer studied the degradation of the RNA with very dilute ribonuclease in order to decide whether the entire strand of RNA is necessary for the infection or whether some active fragments can be obtained During incubation with ribonuclease the decrease of viscosity and optical rotation and the increase in absorption was measured Even a decrease in viscosity of 10 per cent led to a reduction in activity of 65 per cent Therefore the activity is due to the high molecular material as already shown by differential ultracentrifugation The high inactivation rate caused by a low degradation rate means that even slightly degraded particles have no more activity A quantitative calculation reveals that a few breakages perhaps a single breakage induced by ribonuclease lead to the inactivation of the RNA particle Thus by splitting normal to the axis no small active subunits can be obtained That active subunits could be formed by other means cannot be excluded for instance by separating strands if the RNA were poly stranded This evidence is in line with the result that the TMV rod itself if split into shorter segments loses its infectivity and also with the x ray investigation which led to a sensitive volume of the order of the total RNA core

An exact model of the RNA cannot be derived from these studies The simplest assumption would be a 1 stranded helix in which the nucleotides of adjacent turns are fixed by hydrogen bonds In the TMV the pitch of the RNA helix is adapted to the helix formed by the peptide units The length of the virus is defined by the RNA core because by reaggregation of A protein without RNA definite length is not obtained The RNA core has a length of 3 000 Å and the molecular weight corresponds to about 7 000 nucleotides The peptide unit of TMV contains about 140 amino acids so that the length in the fully extended state would be about 490 Å If we assume that the RNA is the template on which the peptide is produced directly we have to explain this large ratio Possibly the RNA has other functions such as the production of enzymes necessary for the protein synthesis Such functions have been observed with the DNA of bacteriophages

CONCLUSION

The investigation of the structure of the TMV virus has also elucidated the function of the structural parts For multiplication only the RNA is important It serves as genetic material in the same way as the DNA of the bacteriophages and transforming principles The properties of the protein produced in the cell are defined by the properties of the RNA However the protein influences the shape and the stability of the virus It would be interesting to follow these interactions in more detail and to carry out such experiments with other RNA containing viruses

BIBLIOGRAPHY

Braunitzer G Biochim et biophys acta 19 574 1956
Burton K Biochem J 61 473 1955
Colter J S Bird H H and Brown R A Nature 179 859 1957
Commoner B and Lippincott J A Nature 178 767 1956

Commoner B Yamada M Rodenberg S D Wang T Y and Basler F Science 118 529 1953
Dawson L M and MacFarlane A S Nature 161 464 1948
Fraenkel Conrat H J Am Chem Soc 78 882 1956
Fraenkel-Conrat H and Williams R C Proc Nat Acad Sc 41 690 1955
Franklin R E Nature 175 379 1955
—— Nature 177 928 1956
Franklin R E and Holmes K C Biochim et biophys acta 21 405 1956
Franklin R E and Klug A Acta crystallograph 8 555 1955
—— Biochim et biophys acta 23 199 1957
Gierer A Nature 179 1297 1957
Gierer A and Schramm G Nature 177 702 1956
—— Ztschr Naturforsch 11b 138 1956
Ginoza W and Norman A Nature 179 520 1957
Hamers Casterman C and Jeener R Virology 3 197 1957
Harrington W F and Schachman H Arch Biochem 65 278 1956
Harris J I and Knight C A Nature 170 613 1953 J Biol Chem 214 215 1955
Hart R C Proc Nat Acad Sc 41 261 1955
Hershey A D Virology 1 108 1955
Hershey A D and Chase M J Gen Physiol 36 39 1952
Hotz C and Schafer W Ztschr Naturforsch 10b 1 1955
Jeener R Lemoine P and Lavad homme C Biochim et biophys acta 14 321 1954
Kramer E Ztschr Naturforsch 12b 609 1957
Lippincott J A and Commoner B Biochim et biophys acta 19 198 1956
Markham R and Smith K M Parasitology 39 330 1949
Niu C I and Fraenkel Conrat H Arch Biochem 59 538 1955
—— Biochim et biophys acta 16 597 1955
Peters D and Nasemann T Ztschr Naturforsch 8b 547 1953
Schafer W and Zillig W Ztschr Naturforsch 9b 779 1954
Schafer W Zillig W and Munk K Ztschr Naturforsch 9b 329 1954
Schramm G Naturwissenschaften 31 94 1943
—— Ztschr Naturforsch 2b 217 and 249 1947
Schramm G and Braunitzer G Ztschr Naturforsch 8b 61 1953
Schramm G Braunitzer G and Schneider J W Nature 176 456 1955
Schramm G Schumacher G and Zillig W Ztschr Naturforsch 10b 481 1955
Schramm G and Zillig W Ztschr Naturforsch 10b 493 1955
Schuster H Schramm G and Zillig W Ztschr Naturforsch 11b 339 1956
Takahashi W N and Ishii M Am J Botany 40 85 1953
Tamizawa J and Sunakawa S J Gen Physiol 39 553 1956
Van Rysselberge C and Jeener R Biochim et biophys acta 23 18 1957

Studies of Purified Polio Viruses

DR CARLTON E SCHWERDT

I will review some observations made on the purification, identification and characterization of polio virus Types 1 2 and 3 grown in tissue culture As Dr Bawden has pointed out these observations are the result of 3 years of investigation carried out at the Virus Laboratory University of California at Berkeley with the cooperation of Dr Frederick L Schaffer

PURIFICATION

The procedure for purifying polio viruses is relatively simple and can be applied directly to large volumes of infectious tissue-culture fluid (TCF) at refrigeration temperatures The source of virus for these studies was infectious fluid from Maitland type cultures of monkey kidney tissue fragments inoculated with Mahoney MEF 1 or Saukett strains Assays for following the course of purification were carried out by the plaque technic on monolayer cultures of monkey kidney or human amnion epithelial cells

Rather than present in detail the basic purification procedure I shall simply enumerate the essential steps as follows (1) precipitation of the virus from tissue-culture fluid with 15 per cent methanol at pH 4 and elution of the precipitate with a one fiftieth volume of mo NaCl solution at pH 9 (2) 2 extractions w n butanol (3) reprecipitation and elution virus as in Step 1 but without methanol (1 cycle of high and low speed ultracentrifug tion (5) treatm nt with crystalline ribo- a deoxyribonucleases (6) a final cy le of hi and low speed ultracentrifugation

Partially purified virus suspensions prepar in this way were 30 000 to 50 000 times mo concentrated than the original tissue-cultu fluid The average recovery of infectivity yie of partially purified virus specific infectivity a purification factor for all 3 virus types are su marized in Table 113 I should like to poi out in passing particularly to those who a interested in a more potent killed virus vacci that any one or all of these steps can be appli to the purification and concentration of pol viruses on a commercial scale

Further purification of the partially purifi virus concentrates was effected either by mov boundary electrophoresis or ultracentrifugal sed mentation in a sucrose density gradient Th latter procedure which was more efficient us ally fractionated the virus concentrates into clearly discernible components readily harveste

TABLE 113 AVERAGE RESULTS OF BASIC PURIFICATION OF POLIOMYELITIS VIRUS FROM TCF*

VIRUS TYPE	% RECOVERY OF INFECTIVITY	YIELD OF PARTIALLY PURIFIED VIRUS (MG /1 TCF)	SPECIFIC INFECTIVITY†	PURIFICATIO FACTOR‡
1 (Mahoney)	40	18	2.9×10^{14}	130
2 (MEF 1)	100	24	1.2×10^{13}	650
3 (Saukett)	20	11	1.0×10	150

* These a erages rep r ent the arithm t mean of 11 or 12 pu if t n experime t on ea h of the 3 ru stran pr pagated in ti ue cultu e

† Number of plaq e f rm ng un ts (PFU)/Gm p t in

‡ Specifi infectivity of purified concentrate / Specifi infectivity of TCF

Schwerdt C. E Phy al and ch m l char te i ti s of purified p l n y l t s ı us in Cell la Bi l gy Nu l Ac ds and Viruses (Speci l Pub No 5) pp 15 166 New Yo k New York Acad Sc 195

free of one another These components were designated A B C and D in the order of increasing sedimentation rates The major and fastest sedimenting component contained approximately 95 per cent of the infectivity of the original concentrate This component freed of sucrose by dialysis was subjected to physical and chemical characterization studies

IDENTIFICATION OF THE VIRUS PARTICLE

In order to identify the physical particle with which the property of virus infectivity is associated it is necessary to determine the concentration of the characteristic physical particle present in a virus preparation by some procedure such as quantitative electron microscopy and to correlate this particle concentration with the infectivity titer The ingenious spray-droplet technic for counting physical particles by electron microscopy developed by Backus and Williams in 1950 has been applied successfully to this problem in the case of the polio virus particle

This method was first applied to polio virus by Bachrach and Schwerdt who found that Lansing polio virus partially purified from brain and spinal cord pools of infected cotton rats consistently required about 21 000 physical particles per 50 per cent lethal dose in cotton rats In the more recent work on polio viruses purified from tissue-culture fluid stored for 6 months to 1 year and assayed by the plaque technic on monkey kidney monolayer cultures the ratio of particles to infectious units (plaques) ranged from 1 000 to 2 500 This ratio has been reduced still further as illustrated in Table 114 when in a study with Fogh virus was freshly harvested from monolayer cultures of infected cells and assayed for plaques on monolayer cultures of human amnion cells Under these latter conditions approximately 30 to 35 physical particles of Mahoney virus were found to be equivalent to 1 plaque

Ideally the ratio of characteristic physical particle to infectious unit should be 1 since the formation of a plaque in tissue culture is probably initiated by 1 infectious particle This ideal ratio may not have been achieved in any preparation of polio virus as yet because some of the physical particles may have been synthesized in an incomplete form or may have suffered inactivation during manipulation However virtually

FIG 185 Type 2 (MEF 1) polio virus particles air dried from an ammonium acetate suspension Particles at the edge of close packed arrays or individual particles are slightly flattened and appear to be larger than those deeper within the arrays The large white sphere is a polystyrene latex reference particle and is 260 mμ in diameter

TABLE 114 PHYSICAL PARTICLE TO PLAQUE RATIOS OF POLIOMYELITIS VIRUS FROM FRESHLY HARVESTED INFECTIOUS TCF

VIRUS TYPE	PHYSICAL PARTICLE/PFU
1 (Mahoney)	35
	38
	56
	34
	28
	41
	[illegible]
	32
2 (MEF 1)	60
3 (Saukett)	119

Plaq f rm g u t

S hw d C. E Phy l d ch m l h r t x f p fied pol my l t r Cell l B l gy N l c A l a d V r se (Special l b N 5) pp 157 166 N w Y k N w Y k Ac l S 195

Studies of Purified Polio Viruses

DR. CARLTON E. SCHWERDT

I will review some observations made on the purification, identification and characterization of polio virus Types 1, 2 and 3 grown in tissue culture. As Dr. Bawden has pointed out, these observations are the result of 3 years of investigation carried out at the Virus Laboratory, University of California at Berkeley, with the co-operation of Dr. Frederick L. Schaffer.

PURIFICATION

The procedure for purifying polio viruses is relatively simple and can be applied directly to large volumes of infectious tissue-culture fluid (TCF) at refrigeration temperatures. The source of virus for these studies was infectious fluid from Maitland-type cultures of monkey kidney tissue fragments inoculated with Mahoney, MEF 1 or Saukett strains. Assays for following the course of purification were carried out by the plaque technic on monolayer cultures of monkey kidney or human amnion epithelial cells.

Rather than present in detail the basic purification procedure, I shall simply enumerate the essential steps as follows: (1) precipitation of the virus from tissue-culture fluid with 15 per cent methanol at pH 4 and elution of the precipitate with a one-fiftieth volume of molar NaCl solution at pH 9; (2) 2 extractions with n-butanol; (3) reprecipitation and elution of virus as in Step 1 but without methanol; (4) 1 cycle of high- and low-speed ultracentrifugation; (5) treatment with crystalline ribo- and deoxyribonucleases; (6) a final cycle of high- and low-speed ultracentrifugation.

Partially purified virus suspensions prepared in this way were 30 000 to 50 000 times more concentrated than the original tissue-culture fluid. The average recovery of infectivity, yield of partially purified virus, specific infectivity and purification factor for all 3 virus types are summarized in Table 113. I should like to point out in passing, particularly to those who are interested in a more potent killed-virus vaccine, that any one or all of these steps can be applied to the purification and concentration of polio viruses on a commercial scale.

Further purification of the partially purified virus concentrates was effected either by moving-boundary electrophoresis or ultracentrifugal sedimentation in a sucrose density gradient. The latter procedure, which was more efficient, usually fractionated the virus concentrates into 4 clearly discernible components readily harvested

TABLE 113. AVERAGE RESULTS OF BASIC PURIFICATION OF POLIOMYELITIS VIRUS FROM TCF*

VIRUS TYPE	% RECOVERY OF INFECTIVITY	YIELD OF PARTIALLY PURIFIED VIRUS (MG./1 TCF)	SPECIFIC INFECTIVITY†	PURIFICATION FACTOR‡
1 (Mahoney)	40	18	2.9 × 10[illegible]	130
2 (MEF 1)	100	24	1.2 × 10[illegible]	650
3 (Saukett)	20	11	1.0 × 10[illegible]	150

* These averages represent the arithmetic mean of 11 or 12 purification experiments on each of the 3 virus strains propagated in tissue culture.
† Number of plaque-forming units (PFU)/Gm. protein.
‡ Specific infectivity of purified concentrate / Specific infectivity of TCF

Schwerdt, C. E. Physical and chemical characteristics of purified poliomyelitis virus. In Cellular Biology, Nucleic Acids and Viruses (Special Pub. No. 5), pp. 157-166. New York: New York Acad. Sc., 1957.

free of one another These components were designated A B C and D in the order of increasing sedimentation rates The major and fastest sedimenting component contained approximately 95 per cent of the infectivity of the original concentrate This component freed of sucrose by dialysis was subjected to physical and chemical characterization studies

IDENTIFICATION OF THE VIRUS PARTICLE

In order to identify the physical particle with which the property of virus infectivity is associated it is necessary to determine the concentration of the characteristic physical particle present in a virus preparation by some procedure such as quantitative electron microscopy and to correlate this particle concentration with the infectivity titer The ingenious spray-droplet technic for counting physical particles by electron microscopy developed by Backus and Williams in 1950 has been applied successfully to this problem in the case of the polio virus particle

This method was first applied to polio virus by Bachrach and Schwerdt who found that Lansing polio virus partially purified from brain and spinal cord pools of infected cotton rats consistently required about 21 000 physical particles per 50 per cent lethal dose in cotton rats In the more recent work on polio viruses purified from tissue-culture fluid stored for 6 months to 1 year and assayed by the plaque technic on monkey kidney monolayer cultures the ratio of particles to infectious units (plaques) ranged from 1 000 to 2 500 This ratio has been reduced still further as illustrated in Table 114 when in a study with Fogh virus was freshly harvested from monolayer cultures of infected cells and assayed for plaques on monolayer cultures of human amnion cells Under these latter conditions approximately 30 to 35 physical particles of Mahoney virus were found to be equivalent to 1 plaque

Ideally the ratio of characteristic physical particle to infectious unit should be 1 since the formation of a plaque in tissue culture is probably initiated by 1 infectious particle This ideal ratio may not have been achieved in any preparation of polio virus as yet because some of the physical particles may have been synthesized in an incomplete form or may have suffered inactivation during manipulation However virtually

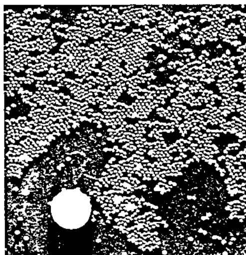


Fig 185 Type 2 (MEF 1) polio virus particles air dried from an ammonium acetate suspension Particles at the edge of close packed arrays or individual particles are slightly flattened and appear to be larger than those deeper within the arrays The large white sphere is a polystyrene latex reference particle and is 260 mμ in diameter

Table 114 Physical Particle to Plaque Ratios of P lio iyelitis Virus from Freshly Harvested Infectious TCF

Virus Type	Physical Particle/PFU
	35
	38
	56
1 (Mahoney)	34
	28
	41
	22
	32
2 (MEF 1)	60
3 (Sauk tt)	119

Pl q f m g t

S hw dt C. E Ph l d h m l h t f p fi d pol my l Cell l l l gy N l c A l l V se (Spec l P b N 5) pp 157 166 N w Y k N w Y k A d S l95

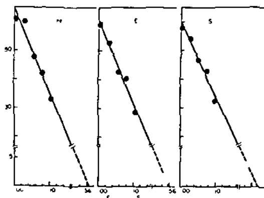


FIG 186 The dry weight density of polio virus particles estimated by plotting the product of sedimentation coefficient and solvent viscosity of highly purified virus suspensions in D O H O mixtures as a function of solvent density The straight line was fitted by least squares and extrapolated to zero sedimentation The dry weight density estimates were 1 57 1 56 and 1 62 for Mahoney MEF 1 and Saukett respectively (Schwerdt C E Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 157 166 New York New York Acad Sc 1957

all the characteristic physical particles may have been active after liberation by the infected cell but the existing assay systems may not have been sensitive enough under the conditions employed to detect the infectivity of each particle At present the latter possibility is favored since continued improvement of conditions of virus preparation and assay has reduced the ratio from 21 000 to 30 particles per infectious unit

PHYSICAL PROPERTIES

Both electron microscopic and hydrodynamic methods have been employed to investigate the physical nature of the polio virus particles Electron microscopy has yielded information about the size and shape of the virus particle Analytic ultracentrifugation analysis has provided evidence for the homogeneity of the highly purified virus particles as well as data for the estimation of particle density mass and degree of hydration

When suspensions of highly purified polio virus particles in a volatile diluent are allowed to dry in air on a collodion film the particles align themselves in close packed hexagonal arrays characteristic of uniform spheres Such geometric formations shadow-cast with uranium are illustrated in Figure 185 If the film is presprayed with a dilute suspension of polystyrene latex reference particles which are uniformly 260 mμ in diameter they may be used for the internal calibration of the fields magnification Thus the measurement of linear arrays of virus particles along any of the 3 axes of the hexagonal pattern were made and yielded an average particle diameter of about 27 mμ

Air-dried specimens suffer some distortion due to the surface tension forces exerted at the air water interface The effect which is most noticeable on individual particles and particles at the periphery of a 2-dimensional array manifests itself as a slight flattening of the particles However this artefact is minimized in the interior of a close packed array Therefore an estimate of average particle diameter from 6 or more particles within an array should be close to the true diameter This assumption is supported by data obtained from the measurement of individual virus particles of frozen dried specimens Such particles which suffered no distortion by surface tension forces since the frozen suspending medium was sublimed away also averaged 27 mμ in diameter

An analysis of highly purified preparations of Mahoney MEF 1 and Saukett polio viruses by analytic ultracentrifugation revealed each to be a homogeneous mono-disperse system with corrected sedimentation coefficients ranging between 157 and 160 Svedberg units These values were obtained from dilute preparations containing as little as 0 01 to 0 005 per cent protein and therefore were considered sedimentation coefficients at infinite dilution

The reciprocal partial specific volume or dry weight density of each virus type was determined by analytic ultracentrifugation in various mixtures of D O H O which were 0 14 molar with respect to NaCl By plotting the product of sedimentation coefficient and solvent viscosity as a function of solvent density and extrapolation to zero sedimentation an estimate was made of the solvent density at which no sedimentation would occur This value which is assumed to be equivalent to the dry weight density of the virus particle ranged between 1 56 and 1 62 for all 3 virus types Graphic representation of such data is illustrated in Figure 186

TABLE 115 SOME PHYSICAL PROPERTIES OF POLIOMYELITIS VIRUSES

TYPE	SEDIMENTATION COEFFICIENT	PARTICLE DRY WEIGHT DENSITY	PARTICLE DIAMETER		PARTICLE MASS	MOLECULAR WEIGHT	WATER OF HYDRATION (GM WATER/GM DRY WEIGHT)
			ANHYDROUS	HYDRATED†			
1 (Mahoney)	160‡	1 57	24 0	27 3	1 13 x 10	6 8 x 10⁶	30
2 (MEF 1)	158	1 56	23 9	2 0	1 12 x 10	6 8 x 10⁶	28
3 (Saukett)	157	1 62	23 2	27 2	1 08 x 10	6 4 x 10	37

F m hyd ody m d ta
† F m elect n m scop c d ta
S db rg t
Sch dt C F Phy k l d cl m c l h r t r n f p fi d p l m l t ru C ll l B l gy Nucl A d d V (Sp al P b N 5) pp 15 166 N Y k N Y k A d S 1 57



Fig 187 Crystals of MEF 1 polio virus particles photographed in visible light The largest crystals are about 30μ in length (Schaffer F L and Schwerdt C E Proc Nat Acad Sc 41 1070)

The radius of the anhydrous polio virus particles was estimated by Stokes law making use of the sedimentation coefficient and dry weight density determined experimentally In this calculation it was necessary to assume that the radius of the hydrated form of the sedimenting particle is equivalent to that of the frozen dried virus particles as measured by electron microscopy

The electron microscope and hydrodynamic data as well as the virus particle mass molecular weight and approximate degree of hydration estimated therefrom are summarized in Table 115 On the basis of these results we have concluded that there are no measurable differences in these properties among the 3 virus strains studied and therefore among the 3 immunologic types that they represent

CRYSTALLIZATION

Further evidence for the high degree of homogeneity of the purified virus preparations is their ability to crystallize under appropriate conditions The conditions for crystallization were simply sedimentation of the virus particles by ultracentrifugation and storage of the gel like pellet for several days at 5 C under a small volume of isotonic saline adjusted to pH 5 9

Table 116 Crystallization and Recrystallization of MEF 1 Poliomyelitis Virus

Fractions	Relative Sequence of Fractionation	Specific Infectivity*
Electrophoretic	1	2 2 x 10^9
Density gradient	2	3 1 x 10^9
Crystalline virus	3	3 3 x 10^9
Mother liquor from 1st crystallization	4	3 3 x 10^9
Recrystallized virus	5	3 5 x 10^9
Mother liquor from recrystallization	6	3 4 x 10^9

* Specifi infect ts i l h d as the number of PFU ml /un t OD_{260} where OD_{260} mμ is the optical d n at 260 mμ was len th f r a rus partı le su pen i n l cm thick

Sch er lt C E Phys cal and chem cal characteristics of purifi l poliomyelitis virus in Cellular B l m Nucleic A ids and Vir e (Spec l Pub N 5) pp 1 1 New York New York Acad S 1957

The resulting crystals were small tetragonal prisms with pyramidal ends as illustrated in Figure 187

The largest crystals were approximately 30 μ in length With careful handling this crystalline virus could be washed free of mother liquor dissolved and finally recrystallized without loss of specific infectivity as compared with that of the purified virus suspension before crystallization Table 116 summarizes a typical crystallization and recrystallization experiment with MEF 1 virus strain

The specific infectivity the infectivity per mass of material remains constant throughout the crystallization and recrystallization procedure Dr Schaffer of the Virus Laboratory of the University of California at Berkeley has kindly permitted me to show a photomicrograph of a crystal of Mahoney polio virus which he has prepared since this manuscript was originally written These crystals are perfectly formed as shown in Figure 188 and are perhaps 5 to 6 times larger in their linear dimensions than the MEF 1 virus crystals Dr Russell Steer of the same laboratory has prepared pre shadowed replicas of the cut surfaces of such crystals which demonstrate beautifully their internal structure As you can se in Figure 189 these sections of the crystals reveal uniform particles which are square packed or hexagonally packed

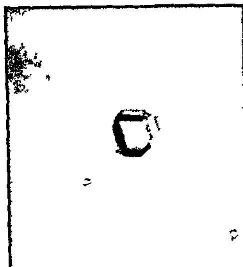


FIG 188 A crystal of Type 1 polio virus (strain Mahoney) Approximate magnification ×50 (Steere I L and Schaffer F L Biochim et biophys acta 28 241)



FIG 189 Electron micrograph of replica of fractured polio virus crystal (strain Mahoney) The packing arrangement of the virus particles within the crystal indicates a space lattice of a face-centered cube Approximate magnification ×75 000 (Steere R L and Schaffer F L Biochim et biophys acta 28 241)

Some preliminary investigations have been made of the chemical nature of the polio virus particles Suspensions of MEF 1 virus particles exhibited a typical nucleoprotein spectrum in the ultraviolet range with an absorption maximum of 260 mμ and a minimum at 241 mμ Quantitative orcinol tests revealed a ribonucleic acid content equivalent to 22 to 30 per cent of the total mass of the virus particle while the Ceriotti indole color reaction indicated little if any detectable deoxyribonucleic acid The absence of deoxyribonucleic acid has been supported by the findings of Dulbecco and Vogt who noted that polio viruses are relatively resistant to inactivation by nitrogen mustard which readily inactivates biologically active deoxyribonucleoproteins

Suspensions of both Mahoney and MEF 1 polio virus particles have been hydrolyzed and quantitatively analyzed for purine and pyrimidine bases by paper chromatography The results of these analyses are summarized in Table 117 Thymine appears to be totally absent The molar distributions of the purine bases of both viruses are similar while those of uracil and cytosine are slightly different Mahoney virus contains more uracil than cytosine on a molar basis The converse is found for MEF 1 virus Confirmation of this small quantitative difference in chemical composition between the 2 viruses requires additional analytic data If it is real it may be the chemical basis at least

TABLE 117 PURINE AND PYRIMIDINE BASES OF PURIFIED MAHONEY AND MEF 1 POLIOMYELITIS VIRUSES

BASES	MOLAR BASE RATIOS TO TOTAL OF 4	
	MAHONEY	MEF 1†
Guanine	1 02	98
Adenine	1 22	1 25
Cytosine	77	96
Uracil	99	81

A r g f 5 lyse F l pu fi n bs se l m n tat n u se d ty gr l t

† O e l F n l p fi t by lect ph etic f ct r ng th m n b l m thod

Schw dt C F Ph al a d h m al h t tic f p f d pxl my l t Cellul r B l gy N l ic 4 f nd V se (Spec [P I N 5) pp 15 166 New Y k N w Y k A d Sc 1957

indirectly for the immunologic differences among the virus types

The MEF 1 particle appears to contain no carbohydrate by anthrone test other than that accounted for by the nucleic acid content As yet analyses have not been made for lipids However the stability of infectivity in the presence of organic solvents and the high dry weight density of the virus particles suggest the absence or low content of this component

In the light of the limited data accumulated so far on the chemical nature of the purified Mahoney and MEF 1 viruses the particles appear to consist of only protein and ribonucleic acid the latter component representing 22 to 30 per cent of their total mass Carbohydrates and deoxyribonucleic acid if present exist in quantities too small to detect by the analytic methods employed

ANTIGENIC PROPERTIES

Mayer observed that highly purified preparations of polio virus are type specific by complement fixation test As antigens these preparations do not cross react with heterotypic sera or with antisera against the constituents of normal *noninfected cells of the type used for tissue* culture propagation of the virus When partially purified virus suspensions are fractionated by the method of sedimentation in a sucrose density gradient as described in this paper the fastest sedimenting component fraction D is found to contain virtually all the infectivity and most but not all of the type specific complement fixing antigen The next slower sedimenting component fraction C on the other hand contains a measurable amount of antigen but no infectivity Mayer has also found that *acute sera from poliomyelitic patients show a higher* complement fixation titer than their respective paired *convalescent* sera with noninfectious fraction C as antigen while the converse is true with infectious fraction D

This suggestion that the fractions C and D are qualitatively different antigens although type specific has been supported by the concurrent findings of Le Bouvier who used our C and D fractions as antigens in the Ouchterlony precipitin test—a technic which involves the double diffusion of antigen and antibody in agar gel Neither fraction formed a precipitate with heterotypic or antinormal cell sera However 2 distinct precipitate zones appeared in the agar gel when both antigens were tested simultaneously against homotypic sera

Fractions C and D differed in physical properties as well as antigenically Electron microscopy revealed greatly flattened poorly delineated particles of low-electron opactiy in fraction C These particles were slightly larger in diameter than the characteristically uniform spherical virus particles found in fraction D Ultraviolet spectrophotometry indicated a marked difference in chemical composition between the 2 fractions The D fraction as noted earlier exhibited a typical nucleoprotein spectrum while the spectrum of fraction C indicated the presence of little or no nucleic acid and I might add that this has been verified recently by chemical analysis

It is not known as yet whether the slower sedimenting particles of fraction C are noninfectious degradation products of once viable virus particles or are noninfectious particles elaborated directly by the infected cell More detailed antigenic analyses of both fraction C and D particles in parallel with physical and chemical studies may help to answer this question as well as shed some light on the mechanism of polio virus replication

SUMMARY

We visualize the virus particles as being fairly rigid uniform spheres 27 mμ in diameter with a high dry weight density ranging between 1 56 and 1 62 and containing about 0 3 to 0 4 Gm of water per Gm of dry weight particle in aqueous suspension The dry weight mass of these particles is about $1\,1 \times 10^{-17}$ Gm An estimate of their molecular weight therefore averages 6 7 million The physical data accumulated so far on all 3 polio virus types *does not* permit the postulation that their respective characteristic particles exhibit detectable physical differences However this does not exclude possible differences in surface charge which still must be investigated by electrophoretic methods

From chemical analyses the physical particles of polio virus Types 1 and 2 appear to consist of only protein and ribonucleic acid the latter constituent comprising 22 to 30 per cent of the particle mass There is no evidence as yet for detectable amounts of deoxyribonucleic acid being present

The purified characteristic virus particles of all 3 types are type specific by complement fixation and by agar gel precipitin tests However there is isolable from partially purified virus preparations a class of particles which are also type specific by the same serologic tests yet antigenically distinct from the virus particles These particles which are noninfectious and differ physically and chemically from the virus particles may offer an approach to the study of polio virus replication

BIBLIOGRAPHY

Bachrach H L and Schwerdt C E Purification studies on Lansing poliomyelitis virus II Analytical electron microscopic identification of the infectious particle in preparations of high specific infectivity J Immunol 72 30 1954

Backus R C and Williams R C The use of spraying methods and of volatile suspending media in the preparation of specimens for electron microscopy J Appl Physiol 21 11 1950

Dulbecco R and Vogt M Biological properties of poliomyelitis viruses as studied by the plaque technique Ann New York Acad Sc 61 90 1955

——— Plaque formation and isolation of pure lines with poliomyelitis viruses J Exper Med 99 167 1954

Fogh J and Lund R O Plaque formation of poliomyelitis viruses on human amnion cell cultures Proc Soc Exper Biol & Med 90 80 1955

Fogh J and Schwerdt C E Physical particle per plaque ratios observed for human poliomyelitis viruses Fed Proc 15 253 1956

Le Bouvier G L Personal communication

Mayer M M Personal communication

Mayer M M Rapp H L Roizman B Klein S W Cowan K M Lukens D Schwerdt C E Schaffer F L and Charney J The purification of poliomyelitis virus as studied by complement fixation J Immunol 78 435 1957

Ouchterlony O Antigen antibody reactions in gels Arkiv Kemi Mineral Geol B26 1 1949

Schaffer F L and Schwerdt C E Crystallization of purified MEF 1 poliomyelitis virus particles Proc Nat Acad Sc 41 1020 1955

——— Studies on the nucleic acid composition of purified preparations of poliomyelitis virus Fed Proc 14 275 1955

Schwerdt C E Physical and chemical characteristics of purified poliomyelitis virus in Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) pp 157 166 New York New York Acad Sc 1957

Schwerdt C E and Fogh J Physical particles per infectious unit observed for poliomyelitis viruses Virology 4 41 1957

Schwerdt C E and Schaffer F L Purification of poliomyelitis viruses propagated in tissue culture Virology 2 665 1956

——— Some physical and chemical properties of purified poliomyelitis virus preparations Ann New York Acad Sc 61 740 1955

Schwerdt C E Williams R C Stanley W M Schaffer F L and McClain M E Morphology of type II poliomyelitis virus (MEF 1) as determined by electron microscopy Proc Soc Exper Biol & Med 86 310 1954

Williams R C A method of freeze-drying for electron microscopy Exper Cell Res 4 188 1953

Electron Microscopy of Tobacco Mosaic Virus

DR ROBLEY C WILLIAMS

INTRODUCTION

The agent causing mosaic disease of tobacco plants has occupied a unique position with respect to biologic chemical and physical experimentation ever since its pathogenic characteristics were first described by Mayer [1] and its filtrability by Iwanowski[18] and by Beijerinck [3] This circumstance is somewhat a matter of historical accident inasmuch as interest in the varied properties of this agent might be expected to result from the unprecedented discovery that it was filtrable conceivably to the relative exclusion of other agents that might have proved to be *as interesting But as time has gone on it* has become apparent that tobacco mosaic virus (TMV) is of uniquely intrinsic importance to those who would like to clarify somewhat the mysteries of the biologic chemical and physical properties of the viruses As well as being the first pathogen to be shown to be transmissible after filtration it was the first to be purified and chemically characterized TMV was also the first virus to be examined by the methods of physical chemistry the first to be observed in the electron microscope and the first to be analyzed by means of x ray crystallography In recent years it has been subjected to elaborate investigation by x ray analysis and electron microscopy in an effort to elucidate its structure The interest in the structure of TMV has been dramatically intensified quite recently by the discoveries that it can be chemically disintegrated into subunits and can be reassembled either as a protein [3] or as a nucleoprotein [9] with a concomitant loss or partial retention of its biologic activity Since it is now known that its nucleic acid moiety alone is infectious [6,8,1] it is small wonder that the question of its detailed structure assumes considerable importance I think it is fair to say that to the virologist tobacco mosaic virus stands in the role of the fruit fly to the geneticist the hydrogen atom to the spectroscopist and the Andromeda nebula to the cosmologist

GROSS MORPHOLOGY

The particles of tobacco mosaic virus are apt objects for investigation by the electron microscopist They are not so large as to be disconcertingly opaque to the electron beam yet they are large enough to afford some hope that their structural details can be detected They are readily purifiable to a high degree thus relieving the microscopist of his usual and proper doubts concerning the identity of the material being observed Since the particles of TMV may be taken apart chemically and reassembled there is the possibility that the electron microscope *may* be *profitably* used in following these fascinating reactions Perhaps most important, there exists a large amount of information about the structure of TMV, drawn from other sources with which the electron microscopist may compare his observations Although the methods of electron microscopy are not applicable in the small molecular domain where the chemist and the x ray crystallographer feel comfortable the direct visualization of somewhat larger structures is useful in affording a check on the implicit expectations arising from experimentation with or indirect observation of the smaller scale units

I shall refer to tobacco mosaic virus in its extracellular form as simple TMV although it may be argued that a virus is acting as a virus *only when it is in its intracellular state* In the former condition an ordinary suspension of TMV may be shown by the electron microscope to consist of particles that fit this rather rough description stiff cylindrical rods with a constant diameter of about 180 Å and with variable lengths (Fig 190) The observation of an apparent nonconsistency of lengths has attracted attention ever since TMV was first photographed [19] The reason for this attention lay initially in the controversy as to whether TMV (and hence perhaps other viruses) could properly be called molecules Clearly if there was no uniformity in length the term was not

an apt one while if there was uniformity the chemists would at least have a factual platform from which to argue for the relevance of the term

DETERMINATIONS OF LENGTH DISTRIBUTION

Extracellular Virus

The history of experimentation designed to demonstrate microscopically whether or not the particles of TMV are uniform in length is a long and confusing one As might be expected different people obtained different answers since there was no attempt made to standardize the conditions of preparation The degree of uniformity of observed length of the particles is dependent on conditions of extraction of the virus from the plant on the mechanical stresses involved in purification on the pH and ionic strength of the suspending medium and on the procedures used in preparing the specimens for microscopy—to name only a few of the pertinent variables Until 1951 the consensus seemed to be that there was a broad peak in the frequency-distribution curve around a length of 300 mμ and that the curve was distinctly skewed toward the shorter lengths A pertinent observation was that the sharpest distributions were obtained subsequent to preparative procedures that were calculated to be the most gentle and followed by suspension in media at pH s near neutrality One should read Bawden s discussion of the earlier evaluations of length distribution [1]

Williams and Steere[33] investigated the problem of uniformity of length of the particles of TMV by use of a method particularly designed to correct for any distortion of length-distribution that might be imposed by the forces of drying inevitable in the ordinary procedures of electron microscopy By using small spray droplets for the deposition of the TMV material they were able to obtain micrographs of droplet patterns in each of which typically there would be 3 or 4 particles of uniform length (or twice this length) and only 2 or 3 shorter fragments After summing the lengths of the fragments and dividing by an integer they found that most of the fragments could be accounted for as uniform length rods that were present initially in the droplets but which were fragmented on drying From these observations they concluded

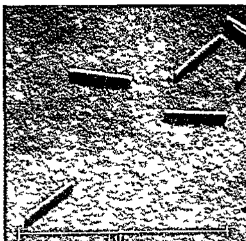


Fig 190 Electron micrograph of particles of purified tobacco mosaic virus (TMV) showing that the virus particles are generally rodlike of uniform diameter and of reasonably uniform length

that *in their preparations* of semipurified material at least 90 per cent of the particles were of uniform length The preparations were made by homogenization of leaves containing TMV as a fairly young infection (about 7 days) followed only by a treatment of the extracted juice at elevated temperature for a few minutes to coagulate the nonvirus protein

Apparently then a suspension of TMV *can* be secured in which almost all the particles are of uniform length The implication of this finding is obvious unless one is prepared to believe that preparative handling can make uniform particles out of those initially nonuniform it must be concluded that TMV exists *within the plant cells* in a highly monodisperse condition Whether or not by various kinds of mishandling one can produce preparations that contain particles of all sorts of lengths has no bearing on this important conclusion such results merely show that sows ears can be made out of silk purses!

Intracellular Virus

More recently it has been possible to show quite directly that at least some of the particles of TMV are of uniform length as they exist within the cell This demonstration has come

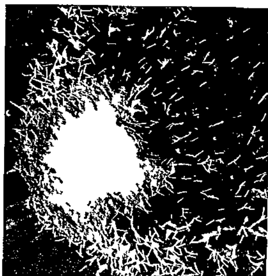


FIG 191 A portion of a partially dissolved inclusion crystal of TMV The individual virus rods are seen wherever the dissolution has been complete

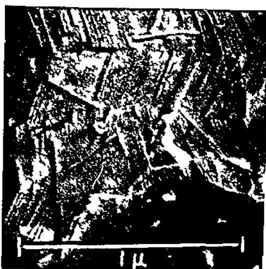


FIG 192 Part of a TMV inclusion *crystal that has been frozen sectioned* and its partially sublimed surface replicated Four tilted layers of closely packed parallel rocks are seen

from an investigation on the identification of the crystalline appearing inclusions found particularly prominently in the hair cells of infected leaves It has been inferred for some years that *these inclusions* were composed primarily of virus particles owing to their optical properties when examined in polarized light By use of these optical methods even the detailed stacking of the presumed virus particles within the crystals has been elaborated [30] But since the crystals have proved to be refractory to removal without complete disintegration and mixing with plant juice the demonstration that they contain primarily virus particles has proved to be frustratingly inconclusive However Steere and Williams [7] were able to remove the crystals intact from the hair cells by methods of freeze drying Rewetting and dissolving the crystals (Fig 191) they were able to count the virus particles by electron microscopy and to assay their biologic titer It was found that substantially *all* of the dry mass of crystal is virus and that the virus dissolved from the crystal has undiminished infectious titer Steere [6] has developed a technic by which such a crystal (within its hair cell) can be frozen transected and the exposed crystal surface replicated for observation by electron microscopy When such a crystal is transected in a plane parallel with the direction of length of the TMV particles within a replica shows the particles as they are stacked in situ They are found in palisades (Fig 192) with the virus rods in each aligned closely parallel Every rod in each palisade is found to have the same length—300 mμ Whether or not the cell makes any selection of rods for inclusion within the crystals is not known of course so from Steere s beautiful observations alone we cannot conclude that *all* the TMV particles within the cells are of uniform length But from this direct type of observation *plus the conclusions of Williams and* Steere [33] it is probably safe to conclude that in an infected leaf substantially all the virus particles are of uniform length

It may seem that I have treated the foregoing subject with an undeserved thoroughness Is it of more than academic interest whether or not the particles of tobacco mosaic virus are of uniform length within plant cells? As we shall see later the question of how long is a particle of TMV is becoming of paramount importance Since the virus may now be taken apart and reassembled the question arises as to the criteria to employ to know whether or not the reassembly is complete One criterion is certainly length (or mass since the cross sectional area is de

monstrably constant) and if it is known that native TMV is uniform in length we are better able to assess physically the excellence of our reassembly operation

LENGTH OF THE INFECTIVE UNIT

It is apparent though that the nub of the question relating to the uniformity of length of TMV has not yet been discussed The question of primary relevance is what must be the length of a particle of TMV in order that it can be *infective* i e what is the length of the *virus* particle? Unfortunately this important question has attracted very little effort directed toward a precise answer However it is possible at this time to indicate some generalities bearing on the question It has been known for years[1] that those preparations of TMV that show by electron microscopy (or by any physical method) a preponderance of quite short rods—of length say less than 100 mμ—are relatively noninfectious In other preparations where the relative numbers of short rods is less the infectivity is greater However if very long rods are artificially produced in the preparation by dropping the pH the infectivity is somewhat lowered By infectivity is here meant a number proportional to the number of lesions produced per unit mass of virus In a general sort of way it may be concluded that infectivity is a maximum for those preparations containing the largest relative abundance of rods of length about 300 mμ It is to be noted that although short rods in a given preparation can be aggregated into longer ones by chemical treatment there is no increase in infectivity

Some work has been done to relate infectivity of TMV with the artificial production of short rods[21] A preparation initially highly infective and composed in good part of rods 300 mμ in length was exposed to sonic disintegration It was found that there is a close relationship between loss of infectivity and the relative numbers of broken rods However the mechanical and chemical effects of sonic disintegration are not thoroughly known and one cannot conclude necessarily that this experimentation proves that the simple breaking of the rods is the sole cause of the lowered infectivity

Hart[16] has done some work that bears on the question of the minimal length of the infective unit of TMV He first found that treatment of TMV with detergent at elevated temperature could be so controlled that the preparations retained about 25 per cent of their initial activity But after exposure of the treated preparation to ribonuclease the infectivity was found to drop further by at least a factor of 10 while the controls dropped by only a factor of ? Since Hart previously had found by electron microscopy that more prolonged treatment with hot detergent laid bare a portion of the RNA of the virus[15] the most likely interpretation of the biologic effects of brief treatment followed by ribonuclease is that the detergent exposed a small portion of the RNA and that the enzyme digested this portion i e that the structural integrity of the entire RNA portion of the virus is necessary for infectivity This conclusion is consistent with the notion that nearly if not exactly a full length rod is the viable unit and that this length is probably the 300 mμ one generally found in abundance in gently prepared extracts It is my personal conviction that rods of native TMV are potentially infective only if they are near to this length but I can offer no quantitative limits on the degree of nearness required

DETAILED STRUCTURAL FEATURES

The general morphology of the rods of TMV as observed by electron microscopy is easily described They are seen to have a most frequent length of 2980 ± 10 Å[33] a diameter of about 180 Å when observed as single particles and a center-to-center separation of 150 Å when measured in packed parallel arrays (The inconsistency implicit in the latter two statements will be discussed subsequently) The general shape is that of a stiff rod with blunt ends On a finer scale of size which might still be amenable to electron microscopic examination there are at least 3 questions to ask what is the cross sectional shape of the virus rod is there any periodic structure along the surface of the rod is there any internal structure? As we shall see the existing answers to these questions are not unequivocal nor are they wholly consistent with the conclusions drawn from x ray analysis of TMV structure

SURFACE STRUCTURE

It might be expected that high resolution electron microscopy of the intact virus particles as

they are dried on the specimen support from purified suspensions would delineate their cross sectional form and surface structure But in order to achieve sufficient electron contrast for an object as small as this to be seen clearly it is necessary to shadow the specimen with some heavy metal such as uranium When this is done certain anomalous features of the electron image appear the consequences of which are certainly frustrating and the causes of which are not wholly certain [31] It is my guess that inasmuch as the shadowing operation is carried out in a vacuum maintained by pumps containing oil the specimen surface becomes coated with tiny (~ 30 Å) droplets of oil prior to the deposition of the shadowing metal As a consequence at this level of size or smaller all surfaces look alike in exhibiting a pebbly structure (the metal shadowed oil droplets) Further there appears to be some migration of the metal-coated oil with the result that light and shade in the shadowed specimen cannot be interpreted to be correlated closely with the angle between the surface shadowed and the direction to the source of evaporating metal Whatever the causes may be it is by now well known that electron micrographs of the surfaces of the particles of TMV do not show any periodicities of structur do the particles exhibit any unique sectional shape (such as a circle a square hexagon) when photographed in the orc way (Fig 193)

In an attempt to obviate one of the diffic mentioned Williams[31] broke particles of into very short lengths (~ 60 Å) by soni integration and photographed these segmei seen end-on It was observed that a large fraction of the short pieces of TMV hexagonal in contour while the others mostly distorted disks Williams assumed artifacts introduced by the vagaries of d would be more likely to produce distorted out of initially perfect hexagons than the way round and concluded that it was likely that the cross sectional shape of the e TMV rod was hexagonal However mor cent results obtained from x ray analysis considerable doubt on this conclusion

Any discussion of the structure of TM revealed by electron microscopy must inc the correlative findings from the x ray l tories The first x ray examination mad regularly oriented gels of TMV[4] showed particles to have a center to-center spacin

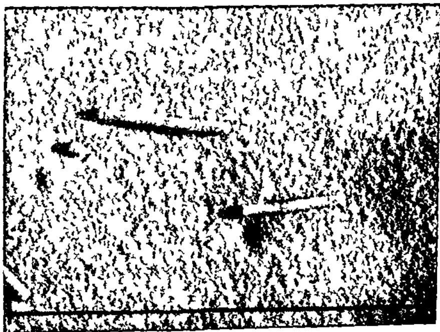


Fig 193 A high resolution electron micrograph of TMV No evidence of periodic surface structure is found

150 A in the dried gel state and also that there was some structural repeating unit of 68 A length along the particle axis In more recent years the x ray structure of TMV has been examined quite intensively[10 11 9] The most striking conclusion from this new work is that the virus rod as a whole is a helix with a pitch of 23 A Structural subunits are found roughly corresponding in size to the known chemical subunits[7 13 14] and there are believed to be 16⅓ of these per turn of the helix There is also evidence for deep grooving along the helix the external appearance of the rod would be like that of a rope wound helically around a small cylinder (Fig 194) It is evident from what has been discussed that the electron microscope has failed to disclose the two primary aspects of surface appearance predicted from the x ray analysis a generally circular cross sectional shape of the TMV rod and a periodic 23 A cross-striation due to the grooves The lack of confirmation of the grooving may well be due to the difficulties associated with shadowing as discussed above but as yet there seems to be no way to reconcile the helical structure (with 16⅓ subunits per turn) with the observed hexagonal shape of thin segments of the virus rod

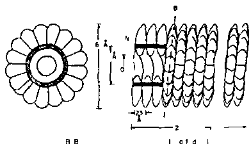


Fig 194 A representation of the helical structure of TMV derived mainly from x ray analysis The cross hatched region shows the nucleic acid at its proper radial position but its disposition along the helix is shown only schematically

Internal Structure

When we consider the *radial* distribution of material within the virus rods there is much better agreement between the x ray and the electron microscopic conclusions The main features of the x ray determination of radial density distribution are (1) from the axis to a radius of 20 A the rod is hollow (2) at 40 A radius there is a region of high x ray scattering believed to be associated with the phosphorus of the viral RNA and (3) the x ray scattering material extends outward to a radius of 95 A [10]

The existence of the predicted hollow cylinder along the axis of the TMV rod has been elegantly confirmed by electron microscopy[17] Huxley found that it was possible to delineate the inner wall of the cylinder by allowing some electron-opaque material such as a phosphotungstic acid solution to enter the cylinder and then to dry leaving a coating of dense metal along its inner wall Micrographs of unshadowed TMV so treated show 2 parallel lines some 50 A apart—exactly the appearance that one would expect if a hollow surface stained cylinder were present

The localization of the RNA within the particle of TMV has been sought for some time by electron microscopists It was not expected that it would be found easily since only 6 per cent of the mass of the particle is RNA and since there is strong evidence that the intact virus rod cannot be chemically stained by any known method But during some observations by Rice *et al* of the effects of freeze-drying on the particles of TMV it was noted that an occasional rod was segmented with the segments lying in a straight line and separated by appreciable intervals Between segments could be seen a fine fiber along the axis of the rod It was speculated that this fiber (~ 40 A in diameter) was the RNA of the virus rod a speculation for which there was no good reason at the time but which later has been shown to be a valid one Schramm and his colleagues[4] have reported that in preparations of TMV partially degraded at high pH there frequently was seen fibrillar material of a diameter such as to suggest that the fibers were RNA Although no chemical test was made to ascertain the validity of this suggestion it was a plausible one since the fibers appeared to be coaxial with the undegraded remnants of the virus rods and since the fibers must be either RNA or else a curiously fibrillar form of partially degraded protein It remained for Hart[15] to demonstrate that the coaxially localized fibers were viral RNA He treated the virus to a detergent solution at ele

vated temperature quickly chilled the material and observed in the electron micrographs that each partially degraded virus rod retained an axially localized fiber at one or both ends (Fig 195) More significantly, an implicit chemical identification of the fibers with RNA was accomplished by treating the partially degraded material with ribonuclease and noting that the fibers then disappeared whereas treatment with other enzymes did not effect their disappearance

There is even further electron microscopic evidence of a somewhat indirect nature for the localization of the RNA within the particles of TMV Schramm[23] has shown that TMV can be partially degraded with alkali to produce particles of weight about 100 000 (the A protein) and free of RNA When such particles are examined in the electron microscope they appear to be disklike but in contrast to the disks or segments produced by sonic disintegration of the native virus they have a central hole about 60 Å in diameter (Fig 196) Similar appearing disks have been photographed by Takahashi and Ishii [8] in preparations of the so-called X protein obtained from the juice of infected plants from which the TMV had been removed by sedimentation The partially polymerized X protein like the A protein is devoid of RNA

It may appear surprising that the determinations of radial density distribution show that x ray scattering material extends to a radius of about 95 Å (diameter = 190 Å) whereas both x ray and electron microscope examination of the virus in packed arrays shows the center to-center separation of the particles (and hence their diameters) to be precisely 150 Å An ingenious way out of this apparent anomaly has been proposed [10] based on the evidence that the rods are deeply grooved The outer radial limit of x ray scattering material would correspond to the ridges of the grooved contour but in packed arrays it would be expected that the rods would fit together ridge to hollow like two machine screws of the same pitch brought together in close parallel contact In this latter case the diameter of a TMV rod would be some sort of mean between the minimal value determined by the depth of the hollows and the maximal value determined by the height of the ridges As a matter of fact there is some indirect electron microscopic evidence for the existence of grooving [3] despite the lack of direct evidence of surface periodicities discussed previously It is found that individual *isolated* particles of TMV are not 150 Å in diameter but are about 180 Å a value that can be thought of as corresponding to the maximal diameter

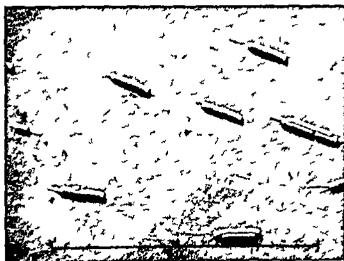


Fig 195 A preparation of TMV that has been heated briefly in a weak detergent solution Part of each rod has been dissolved disclosing the RNA as coaxially localized fibers

determined from curves of radial density distribution

SUMMARY

From the observation of the structure of TMV by methods of chemistry x ray analysis and electron microscopy there emerges the following picture TMV is a nucleoprotein containing some 18 amino acids in its protein portion with its 6 per cent of nucleic acid of the ribose type The protein may be disassembled to an apparently homogeneous subunit with a molecular weight of about 17 000 presumably these are the building blocks Evidently the chemical subunits also have a structural significance since their presence is shown in the x ray patterns where they appear as the fundamental unit in the helical stru ture The protein portion of the virus is assembled in the form of a helix with 49 subunits in 3 turns and the pitch of the helix is 23 Å The exterior of the helix is believed to be deeply grooved The length of the most commonly observed virus particle is 2 980 Å while the diameter (discussed above) is either about 180 Å or 150 Å depending on whether the isolated or the packed virus is considered The central portion of the virus rod is a hollow cylinder 20 Å in radius Immediately outside this hollow tube is a layer of protein in a structural array not known but at a radius of 40 Å there is believed to be resident the phosphorus atoms of the RNA The long-chain RNA molecules are certainly known to be localized coaxially with the rod but the detailed configuration assumed by the RNA is not known There is only protein between the radial locus of the RNA and the outer edge of the rod The cross sectional shape is uncertain but is probably generally circular although not smoothly so When we reflect that almost nothing was known about the structure of TMV 10 years ago except for the observations that it was a nucleoprotein containing 6 per cent RNA and that it was rod like in shape with a diameter of 150 Å we can appreciate that within the last decade this small corner of the virus world has received considerable illumination

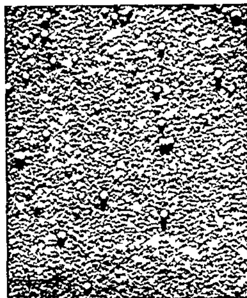


FIG 196 Small particles of protein that come from detergent treated TMV after precipitation of the RNA Most of these are very short cross sections of the initial virus rods and contain central holes These holes are believed to reflect the absence of the virus RNA

REFERENCES

1 Bawden F C *Plant viruses and virus diseases in* Chronica Botanica Waltham Mass Chronica Botanica Co 1950
2 Bawden F C Pirie N W Bernal J D and Fankuchen I Nature 138 1051 1936
3 Beijerinck M W Verh Akad Wet Amsterdam 6 1 1898
4 Bernal J D and Fankuchen I J Gen Physiol 25 111 1941
5 Caspar D L D Nature 177 928 1956
6 Fraenkel Conrat H J Am Chem Soc 78 887 1956
7 Fraenkel Conrat H and Singer B J Am Chem Soc 76 180 1954
8 Fraenkel-Conrat H Singer B and Williams R C *The Chemical Nature of Heredity* Presented at the McCollum Pratt Conference June 1956
9 Fraenkel Conrat H and Williams R C Proc Nat Acad Sc 41 690 1955
10 Franklin R E Nature 177 928 1956
11 Franklin R E and Klug A Biochim et biophys acta 19 403 1956
12 Gierer A and Schramm G Nature 177 702 1956
13 Harris J I and Knight C A J Biol Chem 214 215 1955
14 ——— Nature 170 613 1952

vated temperature quickly chilled the material and observed in the electron micrographs that each partially degraded virus rod retained an axially localized fiber at one or both ends (Fig 195) More significantly an implicit chemical identification of the fibers with RNA was accomplished by treating the partially degraded material with ribonuclease and noting that the fibers then disappeared whereas treatment with other enzymes did not effect their disappearance

There is even further electron microscopic evidence of a somewhat indirect nature for the localization of the RNA within the particles of TMV Schramm [3] has shown that TMV can be partially degraded with alkali to produce particles of weight about 100 000 (the A protein) and free of RNA When such particles are examined in the electron microscope they appear to be disklike but in contrast to the disks or segments produced by sonic disintegration of the native virus they have a central hole about 60 Å in diameter (Fig 196) Similar appearing disks have been photographed by Takahashi and Ishii [5] in preparations of the so called X protein obtained from the juice of infected plants from which the TMV had been removed by sedimentation The partially polymerized X protein like the A protein is devoid of RNA

It may appear surprising that the determinations of radial density distribution show that x ray scattering material extends to a radius of about 95 Å (diameter = 190 Å) whereas both x ray and electron microscope examination of the virus in packed arrays shows the center to-center separation of the particles (and hence their diameters) to be precisely 150 Å An ingenious way out of this apparent anomaly has been proposed [10] based on the evidence that the rods are deeply grooved The outer radial limit of x ray scattering material would correspond to the ridges of the grooved contour but in packed arrays it would be expected that the rods would fit together ridge to hollow like two machine screws of the same pitch brought together in close parallel contact In this latter case the diameter of a TMV rod would be some sort of mean between the minimal value determined by the depth of the hollows and the maximal value determined by the height of the ridges As a matter of fact there is some indirect electron microscopic evidence for the existence of grooving [3] despite the lack of direct evidence of surface periodicities discussed previously It is found that individual *isolated* particles of TMV are not 150 Å in diameter but are about 180 Å a value that can be thought of as corresponding to the maximal diameter



Fig 195 A preparation of TMV that has been heated briefly in a weak detergent solution Part of each rod has been dissolved disclosing the RNA as coaxially localized fibers

determined from curves of radial density distri bution

SUMMARY

From the observation of the structure of TMV by methods of chemistry x ray analysis and electron mi roscopy there emerges the following picture TMV is a nucleoprotein containing *some 18 amino acids in its protein portion* with its 6 per cent of nucleic acid of the ribose type The protein may be disassembled to an appar ently homogeneous subunit with a molecular weight of about 17 000 presumably these are *the building blocks* Evidently the chemical subunits also have a structural significance since their presence is shown in the x ray patterns where they appear as the fundamental unit in the helical structure The protein portion of the *virus is assembled in the form of* a helix with 49 subunits in 3 turns and the pitch of the helix is 23 Å The exterior of the helix is be lieved to be deeply grooved The length of the most commonly observed virus particle is 2 980 Å while the diameter (discussed above) is either about 180 Å or 150 Å depending on whether the isolated or the packed virus is con sidered The central portion of the virus rod is a hollow cylinder 20 Å in radius Immediately outside this hollow tube is a layer of protein in a structural array not known but at a radius of 40 Å there is believed to be resident the phos phorus atoms of the RNA The long-chain RNA molecules are certainly known to be localized coaxially with the rod but the detailed configu ration assumed by the RNA is not known There is only protein between the radial locus of the RNA and the outer edge of the rod The cross sectional shape is uncertain but is probably generally circular although not smoothly so When we reflect that almost nothing was known about the structure of TMV 10 years ago except for the observations that it was a nucleoprotein containing 6 per cent RNA and that it was rod like in shape with a diameter of 150 Å we can appreciate that within the last decade this small corner of the virus world has received consider able illumination

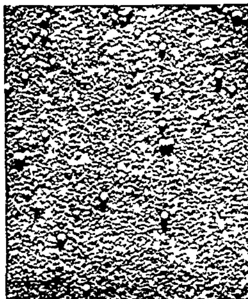


Fig 196 Small particles of protein that come from detergent treated TMV after precipitation of the RNA Most of these are very short cross sections of the initial virus rods and contain central holes These holes are believed to reflect the absence of the virus RNA

REFERENCES

1 Bawden F C Plant viruses and virus dis eases *in* Chronica Botanica Waltham Mass Chronica Botanica Co 1950

2 Bawden F C Pirie N W Bernal J D and Fankuchen I Nature 138 1051 1936

3 Beijerinck M W Verh Akad Wet Am sterdam 6 1 1898

4 Bernal J D and Fankuchen I J Gen Physiol 25 111 1941

5 Caspar D L D Nature 177 928 1956

6 Fraenkel-Conrat H J Am Chem Soc 78 882 1956

7 Fraenkel Conrat H and Singer B J Am *Chem Soc 76 180 1954*

8 Fraenkel Conrat H Singer B and Wil liams R C The Chemical Nature of Heredity Presented at the McCollum Pratt Conference June 1956

9 Fraenkel-Conrat H and Williams R C Proc Nat Acad Sc 41 690 1955

10 Franklin R E Nature 177 928 1956

11 Franklin R E and Klug A Biochim et biophys acta 19 403 1956

12 Gierer A and Schramm G Nature 177 702 1956

13 Harris J I and Knight C A J Biol Chem 214 215 1955

14 ——— Nature 170 613 1952

5 Hart R G Proc Nat Acad Sc 41 261 1955

6 —— Virology 1 402 1955

7 Huxley H E First European Regional Conference on Electron Microscopy 1956

8 Iwanowski D Bull Acad Imp Sc St Petersburg 35 67 1892

9 Kausche G A Pfankuch E and Ruska E Naturwissenschaften 27 292 1939

0 Mayer A Landwirtschaften Vers Stat 32 451 1886

1 Oster G J Gen Physiol 31 89 1947

2 Ri e R V Kaesberg P and Stahmann M A Biochim et biophys acta 11 337 1953

3 Schramm G Ztschr Naturforsch 2b 249 1947

4 Schramm G Schumacher G and Zillig W Nature 175 549 1955

25 Stanley W M Science 81 644 1935

26 Steere R L In Press

27 Steere R L and Williams R C Am J Bot 40 81 1953

28 Takahashi W N and Ishii M Nature 169 419 1952

29 Watson J D Biochim et biophys acta 13 10 1954

30 Wilkins M H F Stokes A R Seeds A R and Oster G Nature 166 127 1950

31 Williams R C Biochim et biophys acta 8 227 1952

32 —— The Nature of Viruses (A Ciba Foundation Symposium) London Church ill 1956

33 Williams R C and Steere R L J Am Chem Soc 73 2057 1951

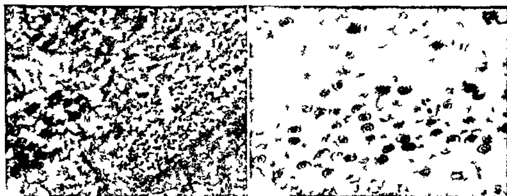
Electron Mıcroscopıc Studıes of Anımal Vıruses ın Thın Sectıons

DR COUNCILMAN MORGAN

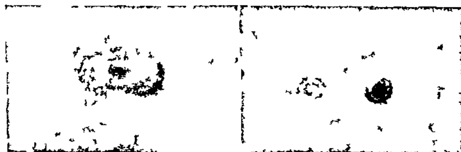
Inıt al attempts to v sual ze ruses n cells employed whole mounts of t ssue cultures Al though ıntracytoplasm c part cles e e encoun tered they could not be dent fied as us th any degree of certa nty The ntroduct on of me hacryla e for embedd ng spec mens enabled relatı ely th n sect ons to be cut on mod fied standard m crotomes Tobacco mosa c rus was the first to be obser ed w hın sect oned cells and shortly the eaf er fowl pox nfluenza ac c nıa and molluscum contag osum vere also seen Because the sec ons were too th ck to perm t adequate resolut on the embedd ng plas t c had to be remo ed w th sol ents The result ng d stort on obl terated mo t of the fıne struc ture of the cell as well as ınternal co nponents of the ruses

Pecogn t on of buffered osm um tetrox de as a su table fixa e (w h subsequent mod hcat on to render ıt ısoton c) and the develoj ment of spec al m croton es perm t ed deta led electron n croscop c study of ruses vı h n cells The fir t v ru es to be exam ned v h these ne v te h n cs vere a c n a ectromelıa and molluscum con ag osum

Rather than re e the numerous excellent papers publ shed dur ng the past few years 5 v uses stud ed ın our labora ory w ll be d s cu ed n so e deta l The follo v ng remarks concern v ork n progress and are ıntended solely



F c 19 (*Left*) ×74 000 (*R ght*) ×39 000



I ıc 198 (*Left*) ×83 000 (*R ght*) ×48 000

15 Hart R G Proc Nat Acad Sc 41 261 1955
16 —— Virology 1 402 1955
17 Huxley H E First European Regional Conference on Electron Microscopy 1956
18 Iwanowski D Bull Acad Imp Sc St Petersburg 35 67 1892
19 Kausche G A Pfankuch E and Ruska E Naturwissenschaften 27 292 1939
20 Mayer A Landwirtschaften Vers Stat 32 451 1886
21 Oster G J Gen Physiol 31 89 1947
22 Price R V Kaesberg P and Stahmann M A Biochim et biophys acta 11 337 1953
23 Schramm G Ztschr Naturforsch 2b 249 1947
24 Schramm G Schumacher G and Zillig W Nature 175 549 1955
25 Stanley W M Science 81 644 1935
26 Steere R L In Press
27 Steere R L and Williams R C Am J Bot 40 81 1953
28 Takahashi W N and Ishii M Nature 169 419 1952
29 Watson J D Biochim et biophys acta 13 10 1954
30 Wilkins M H F Stokes A R Seeds A R and Oster G Nature 166 127 1950
31 Williams R C Biochim et biophys acta 8 227 1952
32 —— The Nature of Viruses (A Ciba Foundation Symposium) London Churchill 1956
33 Williams R C and Steere R L J Am Chem Soc 73 2057 1951

Electron Microscopic Studies of Animal Viruses in Thin Sections

DR COUNCILMAN MORGAN

Initial attempts to visualize viruses in cells employed whole amounts of tissue cultures Although intracytoplasmic particles were encountered they could not be identified as virus with any degree of certainty The introduction of methacrylate for embedding specimens enabled relatively thin sections to be cut on modified standard microtomes Tobacco mosaic viru was the first to be observed within sectioned cells and shortly thereafter fowl pox influenza vaccinia and molluscum contagiosum were also seen Because the sections were too thick to permit adequate resolution the embedding plastic had to be removed with solvents The resulting distortion obliterated most of the fine structure of the cell as well as internal components of the viruses

Recognition of buffered osmium tetroxide as a suitable fixative (with subsequent modification to render it isotonic) and the development of special microtomes permitted detailed electron microscopic study of viruses within cells The first viruses to be examined with these new technics were vaccinia ectromelia and molluscum contagiosum

Rather than review the numerous excellent papers published during the past few years 5 viruses studied in our laboratory will be discussed in some detail The following remarks concern work in progress and are intended solely



Fig 197 (*Left*) ×24 000 (*Right*) ×39 000

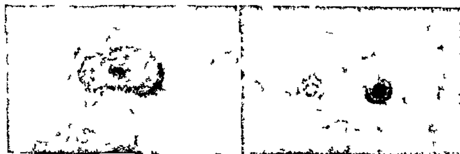


Fig 198 (*Left*) ×83 000 (*Right*) ×48 000

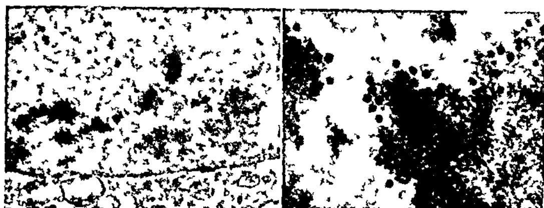


Fig 199 (*Left*) ×9 000 (*Right*) ×38 000

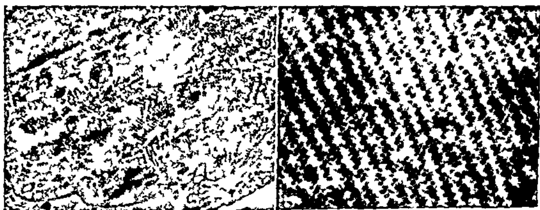


Fig 200 (*Left*) ×10 000 (*Right*) ×25 000

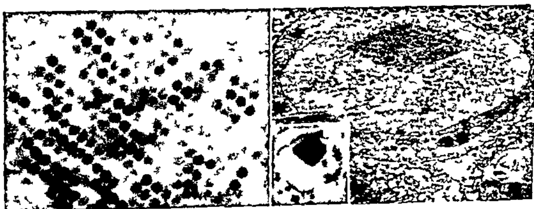


Fig 201 (*Left*) ×50 000 (*Right*) ×3 00

to ind cate the type of nformat on that can be obta ned from th n sect ons I do not presume to suggest that these stud es are defin ti e

HERPES SIMPLEX VIRUS IN THE CHICKEN CHOI IO ALLANTOIC MEMBRANE

Marg nat on of ret ular mater al is the in t al change obser e l with n infected ce ls The nucleus illustrated n F gure 197 left pos esses a d scont nuous l m t ng membrane (M) In the r ght half of the field small part cles a erag ng 400 Å in d ameter and exh b t ng centers of lo v dens ty part ally replace the ma g nated ret cu lum Scattered through the nu lear matr x are larger v ral part les compo ed of an inner body and a dense l m t ng membrane W th n the ytoplasm one p rt cle (P) has a double per ph eral membrane F gure 197 r ght sho vs an ntranuclear aggregate of part cles many of t h ch ha e ncomplete membranes* V rus w h double lim t ng membranes (F g 198 *left*) and a eraging 1,200 to 1,300 Å was en ountered only in the cytoplasm and n the extracellular spa e† Such obser at ons suggest that from marg nated ret c lum v th n the nu leus the rus d fferent ates as a small (400 Å) part le that t increases n s ze and s enclosed by a s ng e membrane (F g 198 *r ght*) that on re ase from the nucleus it acqu res a econd membrane an l that th s form rep e ents th completed nfect ous un t vh ch s encountered n the extr cellular spa e

ADENOVIRUSES IN HELA CELLS

Clusters of viral part les are frequ ntly o ser ed adjacent to ntranuclear aggrega es of den e ret cular mater al (Fig. 199 *left*) (The nuclear memb ane tra er es the lo ver bo der of the field) Th s spac al or en at on of the v rus together ith the poo ly del neated par t cles vis ble in the ret cu lum when exam ned at h gher magn ficat on (F g 199 *right*) sug gests that the v rus d fferent a es fro n the dense ma e ial Types 3 4 and 7 commonly form mult ple ntranucl ar crys als (F g 00 *l ft*) omposed of ral part cles arranged n a cub c body-centere l latt ce The or enta on of the latt ce with respe t to the pla e of sect n w ll largely de erm ne the pa tern obser ed n the m rograph F gure 200 r ght for example llustrates part of a s ng e crystal The l near zones of clearly d fined part les ind cate rus cen al to the plane of sect on whe eas the zones of lo v dens ty and poor de on deno e v rus cut eccentr c lly The part cle a erage 800 Å n d ameter w h a enter-to-center spac ng of app ox mately 650 Å At h gher magn fi at on ? morpholog c types may be d s ngu she l (F g 201 *left*) dense part les th l ttle nternal structure and others less dense w h a central body Such stru tural d fferences may reflect d fferences n fun t on and ra se the quest on whether only one type rep e en s the infect ous un t If th s v ere the case t v ould expla n the

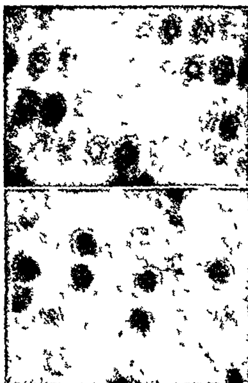


Fig 0? (*Top*) × 130 000
(*Bott m*) × 135 000

* P rt l h h a e poo y d fined be bo n by se sec n ng o ¶ec e loca n n pe t be p f u n h c n qu nt upe mpo n f u e

† P rus w h l b m mb a ha e be n f h l f H La l nfec d w h h ne B ru An x n ev ew of n a al fec d w h h HRE h f l t ea n anal a p t w h d ube Wh h th p n eff t a ff en e n d a n f u o n h f h l ma n o b n e g ed



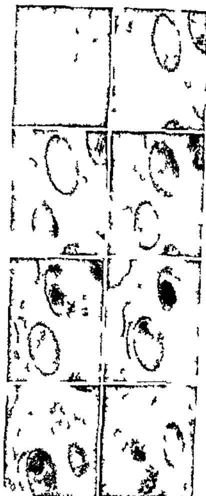


FIG 203 (*Left*) ×120 000
(*Right*) ×52 000

discrepancy bet een part cle counts and infec tivity noted by Hilleman In except onally th n sect on (Fig 202 *top*) the central body is revealed as an area of lo v dens ty enclosed by a th n membrane On the other hand after formal n fixat on a relat ely large and un formly dense central body s obser ed (F g 202 *bottom*)

Adeno iruses undergo no change in structure after release from the nucleus suggest ng that further de elopment does not oc ur in the cytoplas i Chen ical analyses ot th s rus are *not av i lable l ut the crystals of Types 3 4 and* 7 are Feulgen pos t e F gure 201 r ght llus trates the nu leus of a cell conta n ng a rhom bo dal crystal omposed of ral part cles The inset at the lo er left sho s the same nucleus n a contiguous th ck sect on photographed by l ght microscopy after Feulgen sta n ng The crystal is strongly Feulgen pos t e nd cat ng that the v rus conta ns deoxyr l onucle c ac d

FOWL POX AND VACCINIA VIRUSES IN THE CHORIO ALLANTOIC MEMBRANE

Unl ke herpes s mplex and the adeno ruses vacc n a and fo l pox v ruses app ar to develop ent rely v ithin the cytoplasm Figure 203 left sho s 3 part cles of vaccin a One exh b t ng a s ngle l m ng membrane conta ns a dense re t cular body separated from the matrix by an irregular zone of lesser dens ty Ser al sect ons re eal that the otl er part cles undoubtedly have been cut at a le el remo ed from the dense body The 8 consecut e sect ons llustrated n F gure 203 r ght demonstra e (1) that the dense in ternal body ll be transected only when t l es at the appropr ate le el (2) that a part cle cut eccentr cally exh b ts a poorly defined l m t ng memb ane due to ts obl que pos t on w th n the sect on and (3) that s nce the virus vas probably spher cal befo e d stort on l y the m crotome kn fe and measures 2 000 to 2 400 Å in d am eter the a erage sect on th ckness approx ma es 300 Å Fo l pox rus is sho vn n F gure 204 left Al hough its general structure is s m lar to vacc n a at th s stage of de elopment a double l m t ng meml rane is s ble At the left mar gin 2 incomplete iral membranes are cont gu ous to an aggrega e of granular mater al A s m lar though some vhat larger aggregate s



F G 704 (*Left*) ×39 000 (*R ght*) ×24 000

illustrated by Figu e 704 r ght The membranes are ncomplete on the s de adjacent to the gran ules and nearby character stic ral part les are s ble Ser al sect ons through su h s es re ea that the incomplete memb anes are not art fa ts produ ed by the m crotome kn fe Moreo er s m lar s tes ha e been encountered n a d fferent host Figure 705 left shows a sect on through rabbit skin nfected h acc n a rus and aga n granules part ally surrounded by n om p ete membranes are e dent at the upper r ght corner Such obser at ons suggest that these intracytoplasm c s tes e foc of al d fferent a tion

In th s connection Bernhard *et al* obser ed den al s es n the study of the Shope fibroma rus Since a c n a fowl pox ectromel s and the Shope fib oma us possess s m lar n ernal components would har lly be surpr s ng o find that they become d fferentiated n the same manner

Forms cons dered to be ntermed ary n the subse quent cycle of de elopment a e encoun tered nfrequently mpl y ng that the changes occur rather qu ckly F gure 706 left llus trates presumed de elopmental foc (marked by A) of fowl pox rus S udy of s m lar fields nd a es the follo ng sequ nce Dens ret cu lar mater al (B) ncorporated dur ng d ffe ent a t on of the *ral membranes compr ses the dense* nternal l ody (C) Th s body enlarges a l m t ing meml rane forms (D)* and the rema n ng ral matr x d sappears (E) Ser al sect ons of a part cle s m lar to F (F g 0) r *ght*) re eal a central body n the form of a l conca e d sk enclosed by a double meml r ne an l separa ed

* See a so n p e F g u e f

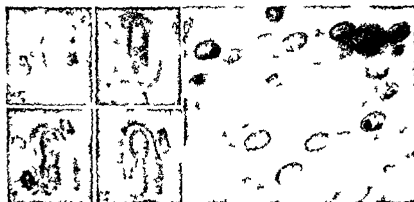


FIG 705 (*Left*) ×33 000 (*R ght*) ×80 000



Fig 206 (*Left*) ×30 000 (*Right*) ×37 000



Fig 207 (*Left*) ×85 000 (*Right*) ×24 000

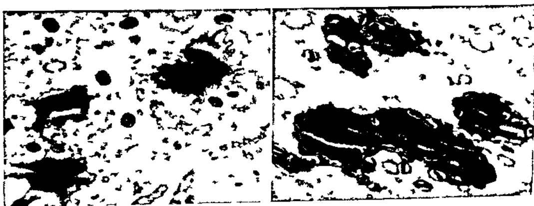


Fig 208 (*Left*) ×18 000 (*Right*) ×16 000

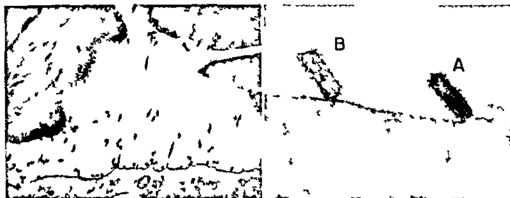


Fig 209 (*Left*) ×10 000 (*Right*) ×97 000

from the peripheral viral membrane by a zone of low density

Vaccinia exhibits slightly different structure at this stage of development a fact not previously recognized As shown by Figure 206 right dense material occupies the zone between the internal body and the peripheral membrane The shape of these particles is best explained by assuming that they are oblate spheroids oriented at random within the section

Vaccinia and fowl pox near the cell surface possess a double limiting membrane In Figure 207 left cytoplasm occupies the right portion of the field the extracellular space is on the left A vaccinia particle appears to be in process of migration through the host-cell wall In Figure 207 right 5 vaccinia particles are visible within the cytoplasm whereas the remainder occupy the intracellular space between contiguous cells † The presumption that this form represents the completed infectious unit is supported by the observation of particles having similar structure in sections of pellets prepared by ultracentrifugation of infective chorioallantoic fluid

Of interest are the aggregates of fowl pox virus at the periphery of dense intracytoplasmic bodies first observed by Eaves and Flewett and assumed by them to represent inclusion bodies We did not see similar structures in our initial studies However osmophilic material is encountered repeatedly in the cytoplasm of a variety of cells and generally accumulates during cellular necrosis Figure 208 left for example shows 3 irregularly shaped dense bodies near particles of vaccinia Flewett's strain of virus (which he was kind enough to send us) appears to destroy the cells relatively slowly therefore large amounts of the dense intracytoplasmic material accumulate frequently incorporating those viral particles which happen to lie in proximity (Fig 208 *right*) Whether such structures constitute inclusion bodies remains to be determined In any case they do not appear to play an essential role in viral development

† The po b ty tha h l s ru d scr bed m ly ep f p d d by fix n n o m um t tr x de h b en ud d by he d tifi a on of m r mo ph g e noo en f r fi n n f m ld h d

INFLUENZA VIRUS ON THE CHORIO ALLANTOIC MEMBRANE

The spherical and filamentous forms of influenza virus develop at the cell surface In Figure 209 left an entodermal cell occupies the lower border Numerous viral particles are in process of release from the free surface Others are attached to 3 red blood cells occupying the upper portion of the field When viewed at sufficient magnification the interior of some filaments appears to be continuous with the host cell cytoplasm (Fig 209 *right* filament A) while the point of attachment of others is revealed as a narrow stalk (Fig 209 *right* filament B) It appears likely that the filaments form by a process of extrusion from the cell wall that subsequently the membrane of the filament encloses the end proximal to the cell and that the intact filament is then released



FIG 210 (*Left*) ×28 000 (*Right*) ×43 000



FIG 211 (Left) ×83 000 (Right) ×90 000

into the chorio allantoic fluid The filaments vary in length as is shown in Figure 210 left Here short filamentous forms line the surface of the host cell whereas a bundle of long filaments extends upward near the right border Such filaments although not exhibiting internal structure possess a dense limiting membrane and a peripheral coat of low density

Frequently the spheres are confined to a portion of the cell surface which is devoid of filamentous forms In Figure 210 right for example the spheres on the left are separated from the filaments on the right At higher magnification (Fig 211 *left*) the spheres although also possessing a limiting membrane and an ill defined peripheral coat can be seen to contain one or several internal bodies The indistinct particles just beneath the cell wall which traverses the field horizontally exhibit incomplete membranes on that aspect nearest the interior of the cell and are believed to represent spheres in process of formation

Figure 211 right illustrates a sphere which is probably central to the plane of a comparatively thick section Its structure is cearly visible

These and similar observations suggest the *following conclusions*

1 The spheres develop in a manner which is different and distinct from that of the filaments

2 The spheres possess one or more internal *bodies whereas the filaments lack visible internal structure*

3 The limiting membrane and the external *coat of both are similar*

If one then makes the assumption that both forms of the virus can act on the surface of red blood cells to cause agglutination but that only the spheres with internal components are capable of initiating infection two recent puzzling observations are clarified Donald and Isaacs em

ploying ultrason c v brat on succeeded in frag ment ng the filaments They noted a r se n hemagglu n n t ter but no apprec able change n nfect ty Burnet obser ed no s gn ficant loss of infectiv ty after destruct on of the fila ments Although the concept that the sphere const tutes the nfect ous un t of influenza rus s cons stent th the a a lal le exper men al data extens e n est ga on ll be necessary to substant ate such a hypothes s

WESTERN EQUINE ENCEPHALOMYELITIS VIRUS IN HUMAN AMNION CELLS*

Small pr mary bod es (c 700 A n d ameter) appear to d fferent ate w th n the cytoplasm of the host cell After release the v rus exh b ts a dense central body averag ng 00 A n d ameter a sharply defined l m t ng membrane of lo v dens ty approx ma ely 350 A n d ameter and a d ffuse per phe al coat (F g 217)



F g 217 ×88 000

CONCLUSION

Exam nat on of mo phology pro des some ns ght nto the manner of ral d fferen a on but s ructure unless correlated v h funct on rema ns stat c If ult mately the mechan sms whereby an mal v ruses de elop are to be under s ood techn cs s m lar to those en ployed so successfully for the study of tol acco mosa c v rus must be adap ed and extended The chem cal nature mmunolog c p opert es and effect on host cells m ght thus be determ ned for d ffer ent structural components

BIBLIOGRAPHY

Banfield W G Bunt ng H S rauss M J and Meln ck J L Electron m c ographs of th n sect ons of molluscum con ag osum Proc Soc Exper B ol & Med 77 843 1951

Bang F B Cellular changes n the ch ck cho r oallanto c *membrane nfected* v h herpes s mplex and vacc n a Bull Johns Hopk ns Hosp 87 511 1950

Bang F B and Gey G O Electron m cros copy of t ssue cultures infected w th the v rus ot Eastern equ ne encephalomyel t s Proc Soc Exper B ol & Med 71 78 1949

Bernhard W Bauer A Harel J and Ober l ng C Les formes intracytoplasm ques du v rus fibromateux de Shope Bull du Cancer 41 423 1954

Black L M Morgan C and Wy koff R W G V sual zat on ot tobacco mosa c rus w th *n infected cells Proc Soc Exper* B ol & Med 73 119 1950

Block D P Morgan C Codman G C Ho e C and Rose H M A correla ed h stochem cal and elec ron m croscop c study ot the ntranuclear crystall ne aggregates of adeno rus in HeLa cells J B ophys & B o chem Cytol 3 1 1957

Burnet F M F lamentous fo ms of influenza rus Nature 177 130 1956

Claude A Porter K P and P kels E G Electron m cros op c study of ch cken tumor cells Cancer Pes 7 421 1947

Donald H B and Isaacs A Some prope t es of nfluenza rus filaments shown by elec tron m croscop c part cle counts J Gen M crob ol 11 325 1954

Ea es G and Flewett T H The structure of to vl pox nclus ons (Boll nger bod es) J Hyg 53 102 1955

Eddy B E and Wyckoff R W (Influenza rus n sect oned t ssues Proc Soc Exper B ol & Med 75 290 1950

Gaylord W H and Meln ck J L De elop mental forms of vacc n a v rus Sc ence 117 10 1953

——— Intracellular fo ms of pox iruses as shown by the electron m croscope (acc n a ectron e a molluscum con ag o m) J Ex per Med 98 157 1953

Harford C G Haml n A Parker E and Pa ens vaay T Electron m croscopy of HeLa cells nfected v th adeno ruses J Exper Med 104 443 1956

Th v nde n e g n at p nt

Hilleman M R Tousimis A J and Werner J H Biophysical characterization of the RI (RI-67) viruses Proc Soc Exper Biol & Med 89 587 1955

Kjellen L Lagermalm G Svedmyr A and Thorsson K B Crystallinelike patterns in the nuclei of cells infected with an animal virus *Nature 175 505 1955*

Low B W and Pinnock P R The packing structure of crystalline RI APC virus J Biophys & Biochem Cytol 2 483 1956

Morgan C Ellison S A Rose H M and Moore D H Serial sections of vaccinia virus examined at one stage of development in the electron microscope Exper Cell Res 9 572 1955

——— Structure and development of viruses as observed in the electron microscope I Herpes simplex virus J Exper Med 100 195 1954

——— Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med *100 301 1954*

Morgan C Howe C Rose H and Moore D H Structure and development of viruses observed in the electron microscope IV Viruses of the RI APC group J Biophys & Biochem Cytol 2 351 1956

Morgan C Rose H M and Moore D H Structure and development of viruses observed in the electron microscope III Influenza virus J Exper Med 104 171 1956

Morgan C and Wyckoff R W G The electron microscopy of fowl pox virus within the chorioallantoic membrane J Immunol 65 285 1950

Newman S B Borysko E and Swerdlow M New sectioning techniques for light and electron microscopy Science 110 66 1949

Palade G E A study of fixation for electron microscopy *J Exper Med 95 285 1952*

Peters D Morphology of resting vaccinia virus Nature 178 1453 1956

Porter K R and Kallman F L Significance of cell particulates as seen by electron microscopy *Ann New York Acad Sc* 54 882 1952

Porter K R and Thompson H P Some morphologic features of cultured rat sarcoma cells as revealed by the electron microscope Cancer Res 7 431 1947

——— A particulate body associated with epithelial cells cultured from mammary carcinomas of a milk factor strain J Exper Med 88 15 1948

Reissig M and Melnick J L The cellular changes produced in tissue cultures by herpes *B virus correlated with concurrent multiplication* of the virus J Exper Med 101 341 1955

Rhodin J Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney Stockholm Aktiebolaget Godvil 1954

Wyckoff R W G The virus of vaccinia in chick embryo membrane Proc Nat Acad Sc 37 565 1951

DISCUSSION

Dr Fraenkel Conrat As Dr Bawden has said there is remarkably good agreement in the field of virology as far as the nucleic acid of TMV is concerned I have something to add to that which will prove that the agreement is slightly better than we thought 6 months ago Dr Schramm has described his nucleic acid as corresponding to the complete complement of nucleic acid in one particle The molecular weight of about 2 000 000 corresponds to the weight of the nucleic acid of one particle whereas preparations that have been made by our detergent technic have been found to resemble more the earlier described preparations of Cohen and Stanley of a molecular weight of about 200 000—one tenth to one twelfth of the whole complement

Recent studies have shown clearly that these two preparations do not differ but that what differs is the way in which you look at them If one ultracentrifuges nucleic acid in 0 0? M phosphate buffer as Schramm has done and if it is good nucleic acid it will act as if it were big If one ultracentrifuges it in very dilute phosphate or water it acts as if it were small And so he was right and we were right and we still do not know who is right because the question is now Is it really big? It refuses to sediment normally only in the absence of ions through charge effects which are poorly understood by the physical chemists Can such enormous differences be due to such charge effects or is it really small but aggregates through the presence of the salt? We still do not know the answer to that question We know that we are both looking at the same animal but we do not *know quite how big the animal is We still* favor the idea that it is small from independent evidence which I do not want to go into now part of which was alluded to by Dr Williams in that we have made nucleic acid from sonically inactivated virus Sonic treatment breaks the rods therefore you should be unable to get complete nucleic acid complement out of any rods They are broken yet we get more activity out of such preparations than one would expect So we still think that the stuff is really small and aggregates in salt but we have little evidence as has the other side about the real nature of the monomer That it may aggregate to bigger things is certain

The study of molecular size in salt has led to other observations which are interesting in that if you keep the nucleic acid at a high ionic strength for any length of time it then loses activity That has been found both by Dr Schramm and by us and we have now found that as it loses activity it also loses its molecular size shape state of aggregation whatever you want to call it It gets again smaller Apparently there is a degradation occurring due to the higher salt concentration and requiring time and temperature This is an effect different from the instantaneous aggregation that I first described

All salts in tenth molar concentration will cause this degradation with the exception of one that we have found so far and that is pyrophosphate It is very interesting because 6 months ago we described how the reconstitution of active virus from nucleic acid and protein occurs remarkably well in pyrophosphate as a buffer We described 6 months ago that you can get 30 to 50 per cent of the activity of the nucleic acid back by reconstitution the same amount of nucleic acid originally in the naked virus Dr Schramm said that if this nucleic acid is really only less active when isolated because it is naked then putting it back in its coat should make it again as active and we do think that we are achieving that to the extent of 30 to 50 per cent of the ideal That is possible only in the presence of pyrophosphate *and it is interesting that pyrophosphate is later* found to be the salt that will not permit the nucleic acid to become degraded So it seems that the reconstituting reaction is one in which you are fighting a losing battle against the rate of decomposition of the nucleic acid You are trying to make rods of protein yet you lose activity of the nucleic acid through its sensitivity to salt and by choosing a salt which is less harmful to the nucleic acid you get highest reconstituted activities

One additional minor point is that recently we have found that the pyrophosphate actually is bound to the nucleic acid when you use radio active pyrophosphate and radioactive phosphate and then dialyze nucleic acid after it has been treated with either of those salts then the pyro phosphate stays with the nucleic acid remarkably more efficiently than the phosphate and it does not dialyze away to the extent of one pyro phosphate residue per nuclear type being held Therefore there is a definite interaction of pyro phosphate with nucleic acid which probably accounts for these observations

Dr Kjellen As we reported in 1955 electron microscopy of thin sections of adenovirus infected HeLa cells revealed patterns of regularly arranged particles in the nuclei In further studies we have tried to correlate the development of specific intranuclear changes with the duration of infection

In cultures infected with Type 5 virus (10^7 ID_{50} 10 cells) the particles and crystal like patterns were often observed to be adjacent to homogeneous grayish zones The first distinct intranuclear changes appeared in some of the sectioned cells 24 hours after infection Characteristic patterns were more frequent in later stages of infections (48 and 72 hours) At 72 hours when many of the cells examined showed almost complete disintegration and light microscopy showed cytopathic degeneration there still remained some cells showing a normal picture on electron microscopy This suggested a lack of synchronization of virus production within the cells It is also possible that not all cells participate in the production of new virus

The suggestion that the intranuclear changes were specifically related to viral reproduction was strengthened by the positive correlation of their development with the rising titer of extracellular virus

Concerning the morphology of the intranuclear particles rounded as well as elongated shapes were observed The diameters ranged from 450 to 700 Å Some of the elongated particles appeared to contain 2 round electron dense structures adjacent to each other

At present we can offer no explanation for the grayish zones observed within the nuclei However they seem to have some bearing on the development of the virus particles

A full account of this work has been published elsewhere

Dr Mattern In the 4 very interesting papers presented this afternoon we have heard described a variety of approaches to the study of several viruses To those interested in the small animal viruses the 2 papers on the chemical and the physical properties of tobacco mosaic virus present a challenge namely that of catching up with the knowledge that has accumulated over more than 20 years of study of this plant virus The paper presented by Dr Schwerdt has indicated that this challenge is being accepted It is along similar lines that our work at the National Institutes of Health is being directed It is my privilege to discuss this work

Coxsackie A 10 virus the etiologic agent of herpangina was grown in suckling mice and purified by methods previously described[6] A virus pellet of high titer obtained by ultracentrifugation and covered with 1 per cent sodium chloride crystallized into dodecahedra The formation of such crystals can be seen in Figure 213 In the upper portion of Figure 213 top left there is a portion of the ultracentrifuged pellet and at the periphery there is early crystallization Figure 213 top right and bottom left and right shows continued crystallization of the same preparation with a concomitant disappearance of the amorphous material

These crystals have obtained maximum dimensions of about 1/10 mm They closely resemble the dodecahedral crystals of tomato bushy stunt virus and one strain of tobacco necrosis virus as obtained by Bawden and Pirie[1] A somewhat different form of crystal was found when the purified virus pellets were covered with 5 per cent ammonium acetate In Figure 214 top left they are seen as 8 sided plates This was not the cleanest preparation we have had but it best shows the shape Bawden[1] has described similar multiple crystal forms for two different plant viruses On low speed centrifugation of such crystals it was found that about 99 per cent of the total infectivity was removed from the liquid phase

Electron microscopy of crystalline preparations was performed utilizing the technic of replication Because of the instability of these crystals on drying their replication—actually pseudoreplication—has not been entirely satis

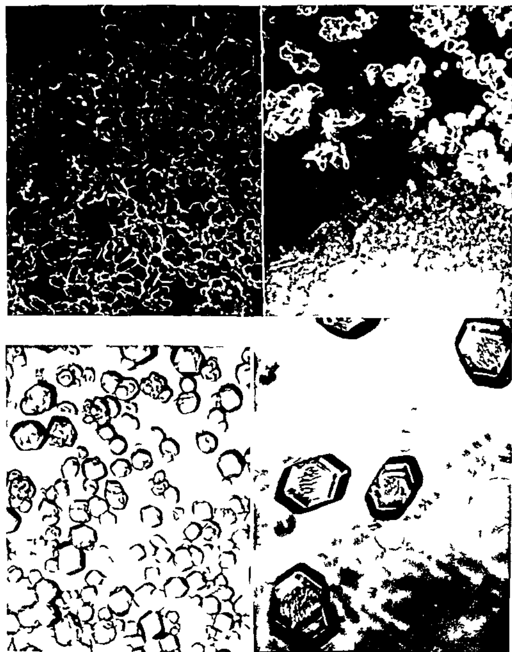


Fig 213 (*Top left*) Portion of ultracentrifuged pellet at periphery early crystallization (*Top right and bottom left and right*) Continued crystallization of same preparation with concomitant disappearance of amorphous material

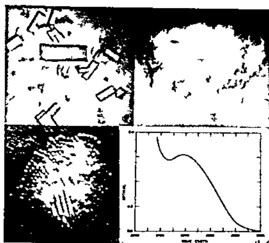


FIG 214 (*Top left*) Showing 8 sided plates (*Top right*) Showing hexagonal arraying at periphery (*Bottom left*) Similar aggregate with an area of rectangular arraying (*Bottom right*) Typical curve of nucleoprotein

factory However both forms of crystal have shown large aggregates of approximately spherical particles 28 mμ in average arrayed diameter Figure 214 top right shows such a preparation with hexagonal arraying at the periphery The center of the mass is impenetrable to the electron beam because of multiple layers of virus Figure 214 bottom left shows a similar aggregate with an area of rectangular arraying The 3 angles in which the particles were found to array by electron microscopy were found to correspond to those angles measured on the crystal faces

In attempting to determine the number of particles necessary to establish infection the volume of several crystal pellets has been estimated to be less than 1/100 ml Such pellets have been found to have a titer of at least 10^{14} LD 50s per ml From the size of the particle it was calculated that about $4\,6 \times 10^{16}$ particles should be contained in a solid ml of virus Thus it appeared that several hundred particles were necessary to establish infection by intraperitoneal titration of suckling mice Intramuscular titration has resulted in a small increase in titer indicating that this ratio is likely to be close to 100 to 1

The ultraviolet absorption spectrum in a solution of recrystallized virus was determined with a spectrophotometer A curve typical of nucleoprotein is seen in Figure 214 bottom right Examination of the same solution in an analytical ultracentrifuge has revealed considerable inhomogeneity despite the ready crystallizability of the virus Schlieren pictures have revealed at least two components The major one with the sedimentation coefficient of about 150 Svedberg units corresponds to the virus particle The other of about 80 units represents a major contaminant of reddish color Companion pictures taken with ultraviolet light revealed the strongly ultraviolet absorbing virus component of about 150 Svedberg units and an additional component of about 40 units It is of interest that Briefs and associates purified the same virus from mouse carcasses by different methods and obtained the same sedimentation coefficient for the virus and also 2 contaminants of 40 and 80 Svedberg units These contaminants have been largely removed by repeated ultracentrifugation and careful washing of the virus pellet to remove the reddish contaminant The sucrose density gradient technic used by Schwerdt and Schaffer[7] should prove to be useful in removing such nonviral contamination

Chemical analysis of this virus has been limited seriously by the small quantities of the purified material available The virus was found to contain about 10 1 per cent total nitrogen by weight This figure is somewhat lower than those reported for most plant viruses and bacteriophase (13 5 to 17 /) but is within the range reported for a number of other purified animal viruses (7 7 to 15 /) Utilizing the orcinol and indole tests for ribose and deoxyribose as described by Ceriotti[3,4] equivocal results were obtained in which a small quantity of ribose was found but no deoxyribose within limits of the test

Three attempts were made to analyze the virus for nucleic acid bases In two tests qualitative results were obtained on hydrolyzing the nucleic acid by a technic described by Markham and Smith[5] By means of paper chromatography spots corresponding in RF to guanine adenine cytidylic acid and uridylic acid were obtained No thymine its deoxyriboside or deoxyribotide have been found These preliminary data are suggestive evidence that this virus contains ribonucleic acid as has been definitely established in the case of the polio viruses

REFERENCES

1 Bawden F C Plant viruses and virus diseases *in* Chronica Botanica Waltham Mass Chronica Botanica Co 1950

2 Briefs A Breese S S Jr Warren J and Huebner R J Physical properties of two Group A Coxsackie viruses when propagated in eggs and mice as determined by ultracentrifugation and electron microscopy J Bact 64 237 1952

3 Ceriotti G Determination of nucleic acids in animal tissues J Biol Chem 214 59 1955

4 ——— A microchemical determination of desoxyribonucleic acid J Biol Chem 198 297 1952

5 Markham R and Smith J D Chromatographic studies on nucleic acids III The nucleic acids of five strains of TMV Biochem J 46 513 1950

6 Mattern C F T and du Buy H G Purification and crystallization of Coxsackie virus Science 123 1037 1956

7 Schwerdt C E and Schaffer F L Purification of poliomyelitis virus propagated in tissue culture Virology 2 665 1956

Dr Melnick Before taking up the main topic of my discussion I would like to show 3 electron micrographs of virus particles taken several years ago The particles that were shown earlier today by Dr Schwerdt and by Dr Mattern are hardly distinguishable morphologically from these A purified preparation of the particles is shown in Figure 215 Like purified polio virus particles they tend to aggregate as shown in Figure 216 and even to form crystalline arrays as shown in Figure 217 When in crystalline formation the particles have a size of 52 mμ As in other virus crystals the particles pack tightly in a hexagonal arrangement with connecting particles in adjacent rows making angles of 120 with each other As indicated the slides are not new and they are not of polio virus particles These preparations of human papilloma virus were obtained 8 years ago and it is gratifying to learn that other human viruses such as those of poliomyelitis and Coxsackie disease are now found to have a similar morphology and similar tendency to form crystalline arrays

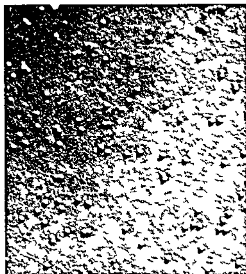


Fig 215 Electron micrograph of spherical virus particles obtained from human papilloma (Melnick J *et al* Ann New York Acad Sc 54 1214)

Ionizing radiation has been used successfully by Pollard and others as a method for determining the internal structure of bacterial and animal viruses A signal advantage of ionizing radiation methods over electron microscopy is the direct relationship which exists between the biologic activity of the unit and the experimental observations There are advantages over ultrafiltration and ultracentrifugation in precision and in that one can determine by ionizing radiation what part of the virus unit is concerned with the function being studied

In the irradiation procedures one measures the loss of biologic function resulting from damage done by ionizations within the unit involved Particles with different densities of ionization along their tracks may be employed Assuming random distribution of hits there will have been an average of 1 hit per sensitive unit at a point where 37 per cent of the virus survives

Deuterons and alpha particles are relatively slow and when fired at the virus particle produce dense ionization along the track The resulting inactivation is proportional to the area of the sensitive unit since at least 1 ionization may be assumed to occur in any virus particle through which a deuteron or alpha particle

may be more complicated The ultrafiltration method gave a much larger size for this virus (140 mμ) than did any of the irradiation methods (48 to 58 mμ) Such differences are taken to indicate a differentiation of function with only the sensitive portion being uniquely essential The relatively close agreement of the data for the measles sensitive unit obtained by electron bombardment with alpha particles and with deuteron measurements indicates that this sensitive unit is approximately spherical

The size of the CF antigenic units of all of the viruses studied fell into the same general range (8 to 13 mμ) This is about the size of a moderately large protein It seems to reside within the virus particle and obviously there is room for many of these CF units within the much larger virus

BIBLIOGRAPHY

Pollard E C Guild W R Hutchinson F and Setlow R B Progress in Biophysics Vol 5 pp 72 108 London Pergamon Press 1955

General Considerations of Viruses

THURSDAY MORNING, JULY 11, 1957

Chairman

SIR MACFARLANE BURNET
Royal Melbourne Hospital
Melbourne

Speakers

DR FRANK L HORSFALL JR
Rockefeller Institute for
Medical Research
New York

DR MAX DELBRÜCK
California Institute of Technology
Pasadena

DR KENNETH M SMITH
Virus Research Unit
Molteno Institute
Cambridge England

DR THOMAS FRANCIS JR
University of Michigan
School of Public Health
Ann Arbor Michigan

Discussants

Moderator DR ANDRÉ LWOFF
Institut Pasteur
Paris

DR W WILBUR ACKERMANN
University of Michigan
School of Public Health
Ann Arbor

DR RENATO DULBECCO
California Institute of Technology
Pasadena

DR WERNER SCHAFER
Max Planck Institut fur Virusforschung
Tubigen Germany

DR IGOR TAMM
Rockefeller Institute for
Medical Research
New York

DR NORTON D ZINDER
Rockefeller Institute for
Medical Research
New York

Opening Comments of Chairman

SIR MAC FARLANE BURNET

One of the problems which plagues me as it has many before me is whether or not it is possible to generalize about viruses at all I have almost joined the majority who think that it is Any type of virus multiplication involves a switch in protein synthesis by the infected host cell From this point of view and from more superficial aspects of the necessity of transfer of a parasite from one cell to another one can legitimately find a basis by which a bacterial virus a plant virus an insect virus and a should we say vertebrate virus can be seen to have some common qualities

Yet I cannot escape the feeling that C16-T2 which is the dominant species of intestinal bacterial virus and influenza A which is the most blatantly successful of human viruses are extraordinarily different things For that matter so are vaccinia influenza A and polio viruses

Therefore it has been a difficult task for the designers of this program to provide what I think they had in mind They wanted speakers who could touch on a wide enough range of topics to give us a sense that the problems of polio are merely a practically important area of an enormously greater field It was necessary to remind us that bacteria plants insects and vertebrates all have their viruses and that in the interaction of parasite and host of virus and susceptible cell there is a microcosm of the whole of functional biology

The solution of those who developed the program is an ingenious one

In this Congress animal viruses must have pride of place and two of our most distinguished animal virologists are to discuss one the multiplication of viruses and the other how that multiplication can be inhibited It is perhaps characteristic of our mid-century position that our concern here is not with the virulence of viruses and how the diseases and deaths that they produce can be prevented but with the search for an understanding of how our empirical successes in the practical field can be related to the basic concepts of biology

By an accident of methodology and opportunity bacterial viruses tend to be considered in the same bracket as animal viruses while plant and insect viruses seem to have a similar illogical affinity So we find Dr Delbruck discussing the genetics of bacterial viruses perhaps the most elegantly logical field of our whole untidy subject We shall find in that contribution a beautiful example of that special quality of the physicist and the mathematician that Delbruck more than anyone else has brought into virology I do not despair of seeing a similar development in animal virology at the hands of Dulbecco my former colleagues Fazekas and Cairns and the new generation

I only regret that when it comes I shall be unable to understand it and that along with my older colleagues who came into virology when it was concerned only with preventive medicine I shall wonder sometimes just what is the bearing of it all on human affairs

Finally Dr Smith is to discuss the latency of viruses a topic that has grown enormously in interest and controversy since Lwoff started looking seriously at lysogeny Dr Smith s examples will come from all the fields of virology and will be of special interest to those of us a majority I fancy who are not expert or knowledgeable in the plant and the insect viruses

Viral Multiplication

DR FRANK L HORSFALL JR

The mechanism of viral multiplication has broad biologic implications for it is part of the more general problem of reproduction It has wide medical implications too for unless viruses multiply in susceptible hosts virus diseases do not occur The papers to be presented at this session of the Conference all will bear on certain aspects of viral multiplication this is evident from their titles which include such terms as genetics latency and inhibition In the present discussion some attention will be directed to important areas in which evidence is inadequate or nonexistent and effort will be made to summarize what has been learned so far in other areas

A virus particle is a tiny object with dimensions of about 250 millimicrons or less that can attach to and infect a susceptible host cell Later in many instances similar objects leave the cell In the interval between attachment and release multiplication occurs in the cell The number of new particles that appear is usually larger than the number that attached to the cell and initiated reproduction

The virus infected cell is the unit of chief interest in relation to multiplication not the virus particle or the host cell separately This unit is in many ways a unique biologic complex with properties different from those of either partner alone and with new potentialities that cannot be recognized from the study of either separately The most arresting capacity of the virus infected cell is the production of new virus particles a feat which neither partner can accomplish by itself The process appears to involve initial disintegration of the infecting particle and in many instances is an unhealthy preoccupation for the host cell Probably if viruses did not cause cell damage they would not have been recognized moreover there would be no virus diseases nor any need for us to assemble in this Conference

The major issue in relation to virus multiplication can be stated simply How is the virus particle reproduced in the infected cell? Apparently this is essentially a biosynthetic problem and a solution probably requires more precise and detailed knowledge of the biosynthesis of nucleic acids proteins and other viral components than now exists

With bacterial viruses much of the phage deoxyribonucleic acid (DNA) but little of the phage protein enters the bacterial cell The important discovery of a new pyrimidine in phage DNA made possible studies on the intracellular synthesis of phage nucleic acid As is now well known phage DNA is synthesized at a different rate than bacterial DNA Recently it was demonstrated that phage DNA synthesis can proceed with little concomitant protein synthesis The sequential synthesis of the two components indicates that phage DNA is not formed inside particles with protein membranes In some as yet mysterious way the spermlike phage particles are finally assembled and are composed largely of DNA and protein both of which are demonstrably different from those of the bacterial host cell

With plant viruses there are now some strikingly similar indications It was recently shown that ribonucleic acid (RNA) purposely separated from toba co mosaic virus particles can initiate reproduction in susceptible plant cells The close analogy to natural events in the initiation of multiplication of phage is fairly obvious Unfortunately a distinctive marker of plant virus RNA has not yet been found so it has not been possible to follow its synthesis directly The synthesis of TMV antigen which is largely protein can be followed but whether the antigen is a precursor of the virus particle or results from abortive biosynthesis is not yet clear How the protein coat and the RNA are brought together to yield the final rodlike particles is still unknown

With animal viruses there are now some indications of a similar kind Recently published data indicate that ribonucleic acid preparations

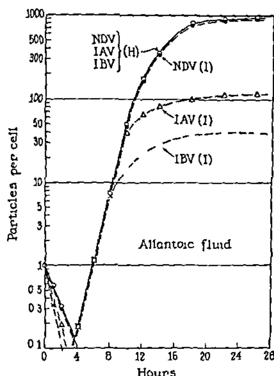


FIG 219 Growth curve of animal viruses

isolated from Ehrlich ascites tumor cells infected with Mengo encephalitis virus can initiate reproduction of that virus The infective component it appears is the RNA itself and not residual contaminating virus particles Thus bacterial *plant and animal viruses have achieved a certain* unity for with each it seems probable that nucleic acid is the component that sets the stage for the production of new virus particles in the infected cell Among animal viruses vaccinia appears to contain DNA but a number of others *are now known to contain* RNA Identifying constituents have not been detected in the nucleic acids so their synthesis cannot yet be followed directly The production of soluble antigenic material which is mainly nucleoprotein can be traced but there is no decisive evidence that such soluble material is a precursor of the final spheroidal virus particles

In contrast to the presently inadequate biosynthetic information there are many data on the dynamics of animal virus multiplication Phage practice and theory have strongly influenced experimentation in this field and a *number of findings are formally similar to those* secured with bacterial viruses Growth curves all tend to show comparable features and in semilogarithmic form closely resemble a ski jump as illustrated in Figure 219 They are flat at the bottom when postadsorptive and at the top and there is a steep slope in between

To examine the kinetics of virus multiplication precisely it seems to be nece sary to count virus particles In the case of animal viruses this is in most instances a difficult problem (Table 119) With vaccinia particle counts by electron microscopy correspond closely to counts of infective units With other animal viruses the correlation has been poor The ratio of counted particles to infective units ranges from 5 1 with Newcastle *35 1 with poliomyelitis and* 100 1 with mumps to even higher values with other viruses By definition the infective property is an essential attribute of a virus particle Particles of similar form composition and antigenic *specificity which do not initiate multipli*cation are still of uncertain significance *Al*though they are clearly by products of multiplication there is no evidence that they contribute or have much relevance to the process Non infective particles perhaps might be designated para viruses they are apparently dead-end objects which are unable to set the stage for reproduction

TABLE 119 ELECTRON MICROSCOPE AND INFECTIVE UNIT COUNTS

VIRUS	COUNTED PARTICLES PER INFECTIVE UNIT	INFECTIVE UNIT PROCEDURE	REFERENCE
Vaccinia	0 7	Pock C A M	Overman and Tamm 1956
Newcastle	5 0	Titration Allantoic	Isaacs and Donald 1955
Influenza	10 0	Titration Allantoic	Isaacs and Donald 1955
Fowl plague	10 0	Titration Allantoic	Isaacs and Donald 1955
Poliomyelitis	35 0	Plaque Amnion	Schwerdt and Fogh 1956
Mumps	100 0	Titration Allantoic	Isaacs and Donald 1955

Approximate values

TABLE 120 PHOTOMETRIC HEMAGGLUTINATION AND INFECTIVE UNIT COUNTS

VIRUS	HEMAGGLUTINATING PARTICLES PER INFECTIVE UNIT	INFECTIVE UNIT PROCEDURE	REFERENCE
Influenza A	3.0	Titration Allantoic	Levine *et al* 1953
Influenza A	1.1	Titration Allantoic	Horsfall 1954
Influenza B	1.6	Titration Allantoic	Horsfall 1955
Swine influenza	1.3	Titration Allantoic	Horsfall unpublished
Newcastle	1.8	Titration Allantoic	Horsfall 1955
Mumps	7.1	Titration Allantoic	Horsfall unpublished

Counts of hemagglutinating particles from erythrocyte sedimentation by photometric means (Table 120) show good correspondence to titration estimations of infective units with various influenza and Newcastle viruses but are poor with mumps. Such counts are based on a biologic character much more distinctive than the particle morphology of animal viruses and give closely reproducible values. With influenza virus a fairly good correlation between them and electron microscope counts also has been secured.

Some of the difficulty in counting infective units is the striking instability of the infective property of various animal viruses (Table 121). The rates of thermal inactivation appear in most instances to be first order. The infective half life at 35° C ranges from about 12 minutes with pneumonia virus of mice (PVM) and 85 minutes with mumps and influenza B to about 13 hours with poliomyelitis and more than 24 hours with vaccinia. Studies on the dynamics of multiplication of unstable viruses are seriously affected by this feature and the yield time curves for infective units may be markedly different from those determined with other particle characters.

Reproduction can be divided for purposes of analysis into 3 phases: latent period, increase period and plateau. After attachment of the infecting virus particles to susceptible cells most particles disappear and cannot be found. How animal viruses penetrate into the host cell is not yet known, though the possibility has been raised that those with a mucoproteinase like influenza may use this aid to enter. Disappearance may be due to disruption of the particle but good evidence for this has been brought forward only with influenza virus.

The usual assumption is that the necessary precursor materials are synthesized during the latent period and it may be that sequential biosynthetic processes are involved as seems to be so with phage. There is not as yet any direct evidence to support this idea but some indirect evidence which is not contrary has been secured with certain benzimidazole derivatives that inhibit RNA synthesis as well as with some amino acid analogues that inhibit protein synthesis.

TABLE 121 INFECTIVE HALF LIFE OF ANIMAL VIRUSES

VIRUS	ENVIRONMENT	INFECTIVE HALF LIFE	REFERENCE
		Hours	
PVM	Mouse lung suspension	0.2	Horsfall and Hahn 1940
Mumps	*Allantoic fluid*	*1.4*	Horsfall unpublished
Influenza B	Allantoic fluid	1.4	Horsfall 1955
Influenza A	Allantoic fluid	2.4	Horsfall 1954
Swine influenza	Allantoic fluid	8	Horsfall unpublished
Newcastle	Allantoic fluid	14	Horsfall 1955
Poliomyelitis	Tissue-culture fluid	13	Fogh and Schaffer unpublished
Vaccinia	C A M suspension	>24	Overman and Tamm unpublished

At 35° C, pH 7.0-7.5

TABLE 122 LATENT PERIOD OF ANIMAL VIRUSES

VIRUS	HOST CELLS	LATENT PERIOD	REFERENCE
		Hours	
W E E	Chick embryo T C	1 2	Rubin *et al* 1955
Influenza	Allantoic membrane	3	Horsfall 1955
Influenza	Mouse lung	<4	Ginsberg and Horsfall 195?
Newcastle	Allantoic membrane	3	Horsfall 1955
Fowl plague	C A M culture	4	Schafer and Munk 1952
Poliomyelitis	HeLa	4	Ackermann *et al*, 1954
Poliomyelitis	Monkey kidney (single)	5	Lwoff *et al* 1955
Vaccinia	C A M	8	Overman and Tamm 1957
PVM	Mouse lung	15	Ginsberg and Horsfall 1951
Mumps	*Allantoic membrane*	*30*	Horsfall unpublished

The latent period (Table 122) ranges from 1 to 2 hours with Western equine encephalomyelitis and 3 to 4 hours with influenza Newcastle and fowl plague up to 8 hours with vaccinia and 30 hours with mumps In some different host cells influenza and poliomyelitis viruses appear to have nearly constant latent periods New infective particles are not found in infected cells during most of the latent period regardless of how they are sought Apparently the final assembly of new particles precedes their release from the cell by only a relatively brief interval

The increase period shows in every instance so far examined a *logarithmic* phase The kinetics correspond to that of an autocatalytic reaction and support the idea that one phase of multiplication is exponential During the logarithmic phase the time to double the number of *particles (Table 123) ranges from about 12* minutes with fowl plague and Western equine encephalomyelitis and 25 minutes with poliomyelitis to 8 hours with vaccinia and 15 hours with mumps

The rate of logarithmic increase appears to be largely independent of the multiplicity over a wide range so long as the inoculum does not exceed about 3 particles per cell When higher multiplicities are used the rate of increase of new particles may be reduced considerably particularly with influenza viruses A similar reduction in the rate of increase may occur when a large number of noninfective particles are added to an infective inoculum that would itself give a relatively low multiplicity With influenza and Newcastle viruses the hemagglutinating particle and infective unit counts correspond closely during the early hours of the logarithmic phase when the multiplicity is low Apparently under these conditions most new particles are infective when they are released from the cell

The release of new particles from infected *cells in a number of instances seems* to be gradual and continuous in contrast with the sudden release of phage on lysis of the infected bacterium Release can continue for many hours after the concentration of virus particles has

TABLE 123 DOUBLING TIME OF ANIMAL VIRUSES

VIRUS	HOST CELLS	DOUBLING TIME	REFERENCE
		Hours	
Fowl plague	C A M *culture*	*0 2*	*Schafer* and Munk 1952
W E E	Chick embryo T C	0 2	Rubin *et al* 1955
Poliomyelitis	HeLa	0 4	Ackermann *et al* 1954
Influenza	Allantoic membrane	0 7	Horsfall 1955
Newcastle	Allantoic membrane	0 7	Horsfall 1955
Influenza	Mouse lung	2 5	Ginsberg and Horsfall 1952
Vaccinia	C A M	8 0	Overman and Tamm 1957
PVM	Mouse lung	*8 0*	Ginsberg *and Horsfall* 1951
Mumps	Allantoic membrane	15 0	Horsfall unpublished

During the log ithmic pha

reached nearly maximal levels in the infected tissue The mechanism of virus release is still unknown but there are numerous indications from electron microscopy that the surface of the releasing cell does not appear to be entirely normal To what extent filament formation as with influenza virus or localized cell surface lysis may contribute to the release of new particles is uncertain

A concentration plateau often follows the increase period and with some viruses this may persist for many hours or some days During the plateau period the concentration of virus particles may remain relatively constant even when measured by infective units This may be the result of a near balance between continuing multiplication release and inactivation of new virus particles Because of the rapid rate of thermal inactivation of a number of animal viruses spontaneous inactivation in vivo can be an important feature in kinetic studies

The yield of infective units per cell at the beginning of the concentration plateau (Table 124) ranges from approximately 600 to 2 000 with Newcastle 100 to 1 000 with poliomyelitis and 50 to 500 with influenza viruses to about 10 to 50 with mumps

A remarkable feature of the reproduction of some animal viruses is the effect of multiplicities greater than one on both the dynamics and the yield of infective units (Table 125) This seems to be most striking with influenza viruses including those in swine but is also evident with mumps and fowl plague although not with Newcastle With multiplicities greater than one the rate of logarithmic increase is reduced and the yield of infective units may become very small If the multiplicity is increased to high values the production of infective units may be almost completely inhibited However noninfective particles which apparently are closely similar to the mature virus and cause hemagglutination although they lack the capacity to initiate reproduction are produced in about the expected large numbers Closely similar results are secured also with input mixtures containing a low multiplicity of infective and a high multiplicity of noninfective particles

The available evidence supports the idea that viral multiplication may be dependent on similar biosynthetic mechanisms whether the host cells are animal or plant including bacteria Of special interest are the indications that the infecting virus particle loses its identity and is disrupted in the process of initiating reproduction in the infected cell The concept that virus

TABLE 125 MULTIPLICITY AND INFLUENZA VIRUS REPRODUCTION

Multiplicity of Swine Influenza Virus	Yield	
	Hemagglutinating Particles Per Cell	Infective Units Per Cent
0.1	220	70
0.5	150	17
1.0	340	5
2.5	350	1.7
5.0	890	0.7
10.0	650	0.3

At 24 29 hour

TABLE 124 YIELD OF ANIMAL VIRUSES

Virus	Host Cells	Approximate Yield Infective Units per Cell	Reference
Newcastle	Allantoic membrane	600–2 000	*Horsfall 1955*
Influenza A	Allantoic membrane	200–500	*Horsfall 1955*
Influenza B	Allantoic membrane	50–[illegible]00	*Horsfall 1955*
Swine Influenza	Allantoic membrane	150–300	Horsfall unpublished
W E E	Chick embryo T C	200–1 000	Dulbecco and Vogt, 1954
Poliomyelitis	Monkey kidney	300–1 000	Dulbecco and Vogt 1954
Poliomyelitis	Monkey kidney (single)	100–200	Lwoff *et al* 1955
Mumps	Allantoic membrane	10–50	Horsfall unpublished

At beginning of plateau

nucleic acid not the intact virus particle orients a number of biosynthetic events in the infected cell is a provocative and stimulating development in biologic theory The indications that certain viral components particularly nucleic acid and protein are produced separately in sequential biosyntheses suggests that viruses are not reproduced as units but in a number of discrete steps The final assembly of the new components into the completed virus particle remains a thoroughly mysterious process with the greatest enigma the acquisition of the capacity to infect another host cell Recent work with some animal viruses raises the possibility that this may be controlled by something comparable to a feed back mechanism in that virus particles themselves in sufficiently high concentration can inhibit one of the last and perhaps one of the most subtle steps in the reproductive process the development of infectivity

BIBLIOGRAPHY

Ackermann W W and Maassab H F Growth characteristics of influenza virus biochemical differentiation of stages of development J Exper Med 100 329 1954

Ackermann W W Rabson A and Hurtz H Growth characteristics of poliomyelitis virus in HeLa cell cultures lack of parallelism in cellular injury and virus increase J Exper Med 100 437 1954

Ada G L and Perry B T The nucleic acid content of influenza virus Australian J Exper Biol & M Sc 32 453 1954

Allfrey V G Mirsky A E and Osawa S Protein synthesis in isolated cell nuclei J Gen Physiol 40 451 1957

Bang F B and Gey G O Comparative susceptibility of cultured cell strains to the virus of eastern equine encephalomyelitis Bull Johns Hopkins Hosp 91 427 1952

Burnet F M Mucoproteins in relation to virus action Physiol Rev 31 131 1951

Cairns H J F Quantitative aspects of influenza virus multiplication III Average liberation time J Immunol 69 168 1952

Commoner B and Rodenberg S D Relationships between tobacco mosaic virus and non virus proteins J Gen Physiol 38 475 1955

Dulbecco R Interaction of viruses and animal cells a study of facts and interpretations Physiol Rev 35 301 1955

Dulbecco R and Vogt M One step growth curve of Western Equine Encephalomyelitis virus on chicken embryo cells grown in vitro and analysis of virus yields from single cells J Exper Med 99 183 1954

——— Plaque formation and isolation of pure lines with poliomyelitis viruses J Exper Med 99 167 1954

Fogh J, and Schaffer F Personal communication from W M Stanley

Fraenkel Conrat H L The infectivity of tobacco mosaic virus nucleic acid *in* Cellular Biology Nucleic Acids and Viruses (Special Pub No 5) p 217 New York New York Acad Sc 1957

Franklin R M Rubin H and Davis C A The production purification and properties of Newcastle disease virus labeled with radiophosphorus Virology 3 96 1957

Gierer, A and Schramm G Infectivity of ribonucleic acid from tobacco mosaic virus Nature 177 702 1956

Ginsberg H S and Horsfall F L Jr Characteristics of the multiplication cycle of pneumonia virus of mice (PVM) J Exper Med 93 151 1951

——— Quantitative aspects of the multiplication of influenza A virus in the mouse lung Relation between the degree of viral multiplication and the extent of pneumonia J Exper Med 95 135 1952

Granoff A Noninfectious forms of Newcastle disease and influenza viruses Studies on non infectious virus occurring within cells that are producing fully infectious virus Virology 1 516 1955

Henle W Developmental cycles in animal viruses Cold Spring Harbor Sympt Quant Biol 18 35 1953

——— Studies on host virus interactions in the chick embryo-influenza virus system J Adsorption and recovery of seed virus J Exper Med 90 1 1949

Henle W Liu O C and Finter N C Studies on host virus interactions in the chick embryo-influenza virus system IX The period of liberation of virus from infected cells J Exper Med 100 53 1954

Herriott R M and Barlow J L Preparation purification and properties of *E coli* virus T2 J Gen Physiol 36 17 1952

Hershey A D Nucleic acid economy in bacteria infected with bacteriophage T2 II Phage precursor nucleic acid J Gen Physiol 37 1 1953

——— An upper limit to the protein content of the germinal substance of bacteriophage T2 Virology 1 108 1955

Hershey A D and Chase M Independent functions of viral protein and nucleic acid in growth of bacteriophage J Gen Physiol 36 39 1952

Hershey A D Dixon J and Chase M Nucleic acid economy in bacteria infected with bacteriophage T2 I Purine and pyrimidine composition J Gen Physiol 36 777 1953

Hershey A D and Melechen N E Synthesis of phage pre ursor nucleic acid in the presence of chloramphenicol Virology 3 207 1957

Hoagland C L Lavin G I Smadel J E and Rivers T M Constituents of elementary bodies of vaccinia II Properties of nucleic acid obtained from vaccinia virus J Exper Med 72 139 1940

Horsfall F L Jr On the consequences of virus reproduction Tr A Am Physicians 68 54 1955

——— On the reproduction of influenza virus Quantitative studies with procedures which enumerate infective and hemagglutinating virus particles J Exper Med 100 135 1954

——— Reproduction of influenza viruses Quantitative investigations with particle enumeration procedures on the dynamics of influenza A and B virus reproduction J Exper Med 102 441 1955

——— Unpublished data

Horsfall F L Jr and Ginsberg H S The dependence of the pathological lesion upon the multiplication of pneumonia virus of mice (PVM) Kinetic relation between the degree of viral multiplication and the extent of pneumonia J Exper Med 93 139 1951

Horsfall F L Jr and Hahn R C A latent virus in normal mice capable of producing pneumonia in its natural host J Exper Med 71 391 1940

Hoyle L and Frisch Niggemeyer W The disruption of influenza virus particles on entry into the host cell J Hyg 53 474 1955

Hoyle L Jolles B and Mitchell R G The incorporation of radioactive phosphorus in the influenza virus and its distribution in serologically active virus fractions J Hyg 52 119 1954

Isaacs A and Donald H B Particle counts of hemagglutinating viruses J Gen Microbiol 12 241 1955

Levine S Puck T T and Sagik B P An absolute method for assay of virus hemagglutinins J Exper Med 98 521 1953

Lwoff A Dulbecco R Vogt M and Lwoff M Kinetics of the release of poliomyelitis virus from single cells Virology 1 128 1955

Morgan C, Ellison S A Rose H M and Moore D H Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med 100 301 1954

Nemes N M and Tamm I Kinetic and biochemical aspects of polio-virus multiplication Anat Rec (in press) 1957

Overman J R and Tamm I Equivalence between vaccinia particles counted by electron microscopy and infectious units of the virus Proc Soc Exper Biol & Med 92 806 1956

——— Multiplication of vaccinia virus in the chorioallantoic membrane in vitro Virology 3 173 1957

——— Personal communication

Pirie N W Anatomy of tobacco mosaic virus Advances Virus Res 4 159 1957

Rubin H Baluda M and Hotchin J E The maturation of western equine encephalomyelitis virus and its release from chick embryo cells in suspension J Exper Med 101 205 1955

Schafer W, and Munk K Eigenschaften tierischer Virusarten untersucht an den Geflugelpestviren als Modell IV Mitt Untersuchungen uber den Ablauf der Vermehrung beim Virus der klassischen Geflugelpest Ztschr Naturforschung 7b 608 1952

Schafer W and Zillig W Über den Aufbau des Virus Elementarteilchens der klassischen Geflugelpest I Mitt Gewinnung physikalisch-chemische und biologische Eigenschaften einiger Spaltprodukte Ztschr Naturforschung 9b 779 1954

Schwerdt C E and Fogh J (quoted by Isaacs A) Particle counts and infectivity titrations for animal viruses Advances Virus Res 4 111 1957

Schwerdt C E and Schaffer F L Some physical and chemical properties of purified poliomyelitis virus preparations Ann New York Acad Sc 61 740 1955

Tamm I and Tyrrell D A J Influenza virus multiplication in the chorioallantoic membrane in vitro Kinetic aspects of inhibition by 5 6-dichloro-1 β D ribofuranosylbenzimidazole J Exper Med 100 541 1954

Wyatt G R and Cohen S S A new pyrimidine base from bacteriophage nucleic acids Nature 170 1072 1952

Zillig W Schafer W and Ullmann S Über den Aufbau des Virus Elementarteilchens der Klassischen Geflugelpest II Mitt Chemische Eigenschaften des Elementarteilchens und seiner Spaltprodukte Ztschr Naturforschung 10b 199 1955

Bacteriophage Genetics

DR MAX DELBRÜCK

Originally I was asked to discuss the genetics of all types of viruses—animal plant and bacterial At my request the topic was limited to bacterial viruses the only ones with which I am familiar from my own research It would be more than hazardous to assume that what has *been found for bacterial viruses should be true* also for animal and plant viruses These 3 classes of viruses are not tied together by any close bonds of relationship Probably they are more closely related to their hosts than to each other Their dissimilarity has found its most eloquent expression in the observation that the bacterial viruses now seem to be the only class whose genetic material is DNA both plant and animal viruses (at present accessible to genetic studies) being devoid of DNA Presumably the plant and the animal viruses utilize their RNA for the storage of genetic information There is certainly little point in extrapolating from one of these nucleic acids to the other

Among the bacterial viruses themselves much diversity exists The most distinct species and the most ubiquitous is the T2 C16 species It has a specialty in its DNA that is absolutely *unique the base cytosine is missing instead it* contains hydroxy methylcytosine (HMC) and this HMC may be glucosylated at the hydroxy position In some strains of this species all the HMC is glucosylated in others only a fraction In some strains the glucosylated fraction actually has diglucose attached Most of the work on phage genetics has been done with representatives of this eccentric species particularly with the strains T2 and T4 and one might question the relevance of these findings even for other species of phages Fortunately such questions can be answered clearly Two other viruses have been studied in enough detail to show their essential similarity in respect to genetics to the T2 C16 species These two other viruses are T1 studied by Bresch and his students in Gottingen and phage lambda a temperate phage studied by Jacob and Wollman at the Institut Pasteur and by Kaiser at the California Institute of Technology In addition some *other phages have been studied less extensively*

Thus what I say makes no claim as to applicability to animal and plant viruses but does claim to be relevant for all phages irrespective *of the peculiarities of their DNA and irrespective* of their temperateness or virulence

Basic for doing phage genetics is the ability to distinguish genetic types and in phage this is done almost exclusively by looking at phage colonies the so-called plaques Such a plaque contains a large number of phage particles but it is initiated by a single particle and the properties of this single particle determine the type of colony Figure 220 illustrates a variety of colony types They differ in diameter sharpness of edge turbidity and even in color in the technic used by Bresch The type of colony depends also on the environmental factors during the formation of the colony such as the *temperature the nutrient conditions* the bacterial strains employed the dyestuffs added to the medium etc These are chosen to suit the case to bring out the sharpest contrast between *the types of colony produced by the various* types of phage Sometimes mutations occur during the growth of the colony giving a mixed or mottled appearance to the plaque and this *mutability also may be used as a characteristic* of the type of phage initiating the plaque In some cases with which we will be concerned particularly the mottled appearance of the plaque is not due to mutations *but is due to* the fact that the original particle is duplex containing 2 opposing genetic elements which segregate on replication of the particle initiating the plaque (*Fig 221*) Such phage *particles* with genetically mixed types are called heterozygotes and they will play an important part in our discussion Principally we must keep in mind that by inspecting the plaques that they produce we can enumerate in a given sample of phage how many particles belong to each

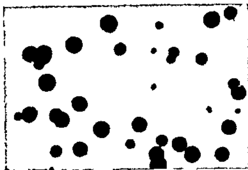


FIG 220 Various plaque type mutants in a phage stock (T4) grown under conditions forcing the incorporation of 5 bromouracil instead of thymine into the DNA



FIG 221 Mottled plaques Such plaques may be due to (a) a phage with a high mutation rate (b) a mixedly infected bacterium (c) a phage heterozygote

type Note particularly that in phage one characterizes practically every infectious particle in the sample because the probability that a particle will form a plaque is under proper conditions always close to 100 per cent

The next point is the source of these various phage types These may be either simply different types collected from various natural sources or types derived by mutation from one standard or wild type For the experiments on recombination the results are ever so much simpler if we use a standard type and a group of mutants derived by mutation from it The crosses between types collected in nature are by no means devoid of interest Particularly the crosses between T2 and T4 studied by Streisinger and Weigle are fascinating because the parental types differ in the degree of glucosylation of the DNA Thus we can study in these cases the inheritance of the chemical characteristics of the genetic material itself These studies reveal some unorthodox features but they are as yet too incomplete to warrant discussion When mutants are used in phage genetics those are in all cases spontaneous mutations A special chemical compound 5 bromouracil an analogue of thymine has recently been shown to be highly mutagenic in phage Litmann and Pardee have shown that phage grown on bacteria which are forced to use this compound instead of thymine to build DNA produce a prodigious amount of mutants Figure 220 which I use to illustrate various phage types is in fact a picture of a 5 bromouracil culture The mechanism of this mutagenesis is under intensive study as yet quite incomplete Since the mutants so produced have not been used in any of the studies of phage genetics they need not concern us further

Figure 222 illustrates a type of experiment that can be done It shows the basic material for studying recombination Bacteria at a high concentration are mixed with a fivefold excess of 2 types of phage the parental types The phage particles attach themselves at random to the bacteria and after 5 minutes most of them are attached Each bacterium has then been infected on the average with 5 particles of each parental type The actual number varies of course from bacterium to bacterium but relatively few bacteria will not have been infected at all by one or the other or even by both parental types After this adsorption period various dilutions are made perhaps including one dilution step through a tube containing specific antiserum to eliminate unadsorbed phage and the final dilution is calculated in advance to be such that a small measured volume contains on the average one fifth of one bacterium A hundred of such small measured volumes are then distributed 1 to each tube Most of these tubes will not contain bacteria or phage About one fifth will contain 1 mixedly infected bacterium and a much smaller fraction will contain 2 or more These tubes are incubated until lysis has occurred At that time the tubes that contained 1 mixedly infected bacterium will now contain the yield of phage produced by this bacterium The tubes then are plated and those that contained a phage yield will after incubation give a plate with plaques 1 plaque for each phage particle in the progeny These plaques are classified according to type and thus the plate tells

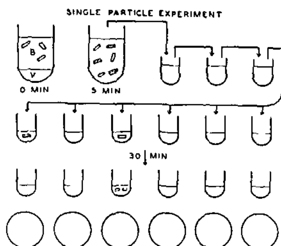


Fig 222 Schematic diagram of a single burst type experiment

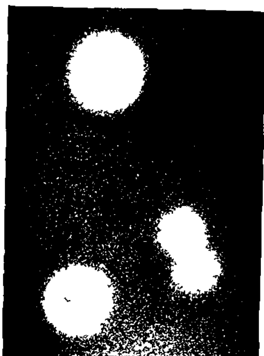


Fig 223 Four plaque types obtained in the progeny of a cross between two mutants (T2r and T2h) of phage T2 Besides the parental types T2r (large turbid) and T2h (small clear) there are present in *the progeny also the two recombinants* $T2r^+h^+$ (small turbid) and T2rh (large clear)

us how many phage particles of each type are produced by this particular bacterium The experiment here described is called a *single burst* type experiment and specifically we have been studying by this technic a genetic cross between the 2 parental types and more specifically we have made the cross under conditions of equal multiple infection (multiplicity 5 for each parent)

The principal findings that have been made by such crosses are the following

1 If the parental types differ by 1 mutational step then the yield contains both these types and no others An exception is the case of phage T3 and its mutants where crosses between 2 parental types differing by only 1 mutational step give a great variety of types in the progeny Obviously in this case the mutational event is more complicated We do not know for certain why this is so and the speculations that have been made to account for this abnormality center around the interactions between phage genetic material and bacterial genetic material (See p 346)

2 If the 2 parental types differ by 2 mutational steps 1 affecting the size and 1 affecting the turbidity of the colony then the yield contains in addition to the 2 parental types the 2 recombinant types as shown in F The proportion in w e reco up may be as r cent 60 per cent fo types ses

the proportion of recombinants may be as low as 01 per cent

3 *The proportion of recombinants is characteristic* for each pair of mutants and it constitutes the principal tool for constructing the genetic map of the phage and its mutants The genetic map as such is not a matter of speculation but simply a concise summary of a large number of experimental results The procedure for arriving at the map may be outlined as follows Assume that we have collected a large number of mutants of a certain standard type of phage We cross these mutants pairwise in all possible combinations and note for each cross the recombination frequency

Then we note first that the mutants may split up into several linkage groups Pairs belonging to the same linkage group have recombination frequencies less than the maximum for the

cross in question while pairs of which the members belong to different linkage groups all have the same maximal recombination frequency The different linkage groups cannot be ordered in relation to each other on any kind of genetic map They sometimes are referred to as the different chromosomes of the phage but this may well be an improper analogy

Then we turn to the individual linkage groups and establish their genetic maps We will describe the mapping procedure in a somewhat unconventional form to bring out what we think is the most essential part namely linear order without introducing the notion of map distance We start with 3 mutants A B and C and look at the recombination frequencies for the pair AB for the pair AC for the pair BC Let us say that the pair AC has the largest recombination frequency Then we must put the mutant B on a straight line somewhere between A and C Now we adjoin to the system a fourth mutant D and follow the same placement rule for each of the 3 triplets formed by combining the new mutant with each of the 3 previous pairs

Thus we proceed adjoining 1 mutant after the other to the system and obtain in the end all the mutants arranged in an ordered series Figure 224 illustrates this procedure for large distances The fundamental fact is that such placements never contradict each other For instance we never find that mutant D according to 1 triplet lies between A and B and according to another triplet lies between B and C while B lies between A and C This is what we mean by saying that we can construct a linear map Note that this definition does not involve the assignment of map distances

In ordinary genetics one proceeds similarly but then finds in addition that the recombination frequencies also can be used to define map distances That means that there exists a simple additivity rule If B lies between A and C then the recombination frequency for the mutants A and C is roughly equal to the sum of the recombination frequencies for the pairs AB and BC This additivity rule holds for ordinary genetics only for small distances for larger distances it is disturbed by the occurrence of multiple recombinations and by the fact that these multiples may not be occurring according to simple statistical rules In phage genetics the

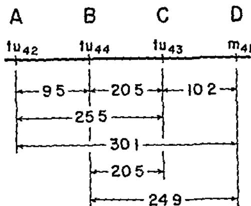


Fig 224 Portion of the genetic map of phage T4 (Doermann A and Hill R Genetics 38 79)

additivity rule is disturbed by two further complications First it has been shown that the phage cross does not correspond to a simple genetic cross Inside a mixedly infected bacterium there occur quite a large number of recombinational events In fact we are dealing with a small population somewhat analogous to the situation in ordinary genetics when we look at the progeny after several generations Another complication which is prominent in phage genetics for small distances will be discussed later The facts and notions here described were put together several years ago into a comprehensive formalism whose premises may be listed as follows

The parental phage particles on infection of the bacterium enter a noninfective so-called vegetative stage In this state they multiply and mate The mating is random with respect to partner If two genetically different vegetative partners mate genetic recombination may occur and the probability that the recombination occurs during the mating act is some function of the distance between the loci assigned to the mutants on the genetic map Specifically we assume that the probability of recombination increases with the distance on the map The totality of the vegetative phage particles is called the pool of vegetative particles From this pool particles are withdrawn at random beginning about midway between the time of infection and the time of lysis and the withdrawn particles become mature infective phage particles

The maturation process is an irreversible process mature particles never return to the vegetative pool Also they do not multiply and do not mate They are quite inert

The justification for this picture has been given in an excellent survey by Doermann and there seems to be no need to go over it again in this report since none of the more recent data lead one to question its validity Great effort has been expended to nail down the physical nature of the vegetative phage particle Is it or is it not simply naked DNA in the form known in extracellular phage? The T2 C16 species is advantageous for such studies because in this case HMC is a natural marker which distinguishes phage DNA from bacterial DNA Moreover the phage DNA synthesized in any particular time interval can be labeled differentially by introducing the radioactive phosphorus isotope P32 for a short interval into the medium Moreover DNA synthesis can be segregated from protein synthesis by suppressing the latter by various means DNA thus synthesized in the absence of protein synthesis is incorporated later into phage after removal of chloramphenicol and resumption of protein synthesis

These experiments demonstrate a separateness of DNA synthesis and protein synthesis and strengthen the idea that the vegetative phage is DNA since this is the principal phage component injected into the bacterium upon infection and the only component which is transmitted to the progeny Beyond this point the physical picture is less certain It was believed earlier that RNA was not involved in phage replication because the RNA seemed to be metabolically inert but now more refined methods have revealed a very small amount of rapidly turned-over RNA and this could be connected with DNA replication Moreover a variety of lines of evidence has led to speculation that the DNA of a phage may be bipartite the two parts being functionally and structurally distinct These problems have been reviewed in great detail recently and will not be treated here except to state that they still await clarification

Returning to the formal picture of phage genetics developed above we note that it leaves several questions of the most fundamental importance unanswered and we will devote the rest of this report to a statement of these questions and to the as yet largely unsuccessful attempts to answer them Foremost is the question as to the interrelation between replication and mating Here we may distinguish 3 notions which have been advocated

1 Replication and mating are quite separate processes recombination is a physical exchange between 2 structures not in the process of replication This notion was implied in the theory of Visconti and Delbruck

2 Mating always involves replication The new replicas recombine in the process of formation Besides this form of replication during mating there is also ordinary replication not involving mating This notion receives support from the structure of heterozygotes as will be seen

3 Replication cannot occur except when 2 particles are mating

Here we need not go into the numerous arguments that have been advanced in favor or against these 3 alternatives Suffice it to say that in our opinion none of the arguments are very strong Perhaps a word of explanation should be added with respect to the last alternative At first sight this seems to be absurd How could replication be restricted to the mated state? In that case single infection should never give any replication and those phage strains which show very little recombination should show very little growth Of course neither of these is true The supplementary hypothesis which has been suggested is that the genetic material of the host bacterium is sufficiently homologous to permit mating and in some cases even recombination of the phage with the host genome Indeed there is very good evidence in some systems of strong interactions and even recombinations between phage and host bacterium but this evidence is by no means conclusive in showing that mating is a necessary condition for replication These questions are discussed very fully in a review article by Stent

Closely related to the alternatives cited is the question whether the recombinational act produces just 1 recombinant or 2 and whether or not the parental types involved in the mating are preserved We can list the alternatives a little more clearly in this manner does the mating produce

(a) 1 particle (a recombinant the parentals being destroyed)

(b) 2 particles (reciprocal recombinants the parentals being destroyed)

(c) 3 particles (2 parentals and 1 recombinant) or

(d) 4 particles (2 parentals and 2 reciprocal recombinants)?

We know perhaps only one thing for certain regarding these alternatives and that is that none of them is quite right The point which is wrong is the idea that the mating produces only parentals and recombinants What it seems to produce instead is something less simple a heterozygote and these we must consider now before we return to the stated questions

First let us describe the phenomenon Among the progeny of mixedly infected bacteria one finds besides the genetically pure particles also genetically mixed particles as shown by the fact that the plaques which these particles produce contain phage particles of two genetic types It has been demonstrated beyond doubt that these mixed particles are not simply two particles stuck together but single particles containing in duplicate at least the particular section of the genetic map responsible for the type difference one copy derived from one parent and one derived from the other parent With respect to this section the particles are therefore heterozygous

We know for sure two more things about the structure of heterozygotes first the heterozygous section is short compared with the whole length of the genetic map second outside the heterozygous section the particles are recombinant that is on one side of it they are pure for the genetic material derived from one of the parents and on the other side of it they are pure for the genetic material derived from the other parent Perhaps we can add one more statement though not with quite the same degree of certainty The frequency with which the heterozygotes turn up is compatible with the idea that for distant markers every recombination act produces in the first instance a particle with a short duplicate section somewhere in between the two recombined markers Does this information suffice for writing down the complete genetic structure of the heterozygote? Unfortunately it does not It leaves open the two alternatives shown in Figure 275 On the left

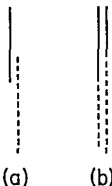


Fig 275 Two alternative structures of phage heterozygotes

(*a*) the particle is described by a map which involves 2 partial replicas 1 of 1 parent and 1 of the other parent with a short region of overlap On the right (*b*) the same particle is described by a duplex structure running the whole length of the genetic map The portions outside the heterozygous region are homozygous representing the genetic material of 1 parent on 1 side and of the other parent on the other side From a purely genetic standpoint we would not know how to distinguish the 2 structures because we have no way of predicting how either of them would behave From a physical standpoint the principal difference is this the structure *a* is duplex only for a short stretch and thus inhomogeneous along its length while the structure *b* is duplex along its entire length and thus physically quite homogeneous along its length One might imagine that it should be easy to distinguish between these 2 alternatives by radiologic methods One might expect perhaps that structure *a* should be inactivated like a single unit exhibiting a 1 hit curve and structure *b* like a double unit exhibiting a 2 hit curve

Now it is certainly true that radiologically phage particles behave as single units but this does not really decide the point because we do not know how intimately the 2 hypothetical units might be associated As a matter of fact we are certain that the genetic material in the mature phage is duplex at least in the sense of the Watson-Crick double helix Therefore this duplicity at least does not show up radiologically We could well imagine that there are even more complex duplex structures which do not show up radiologically Thus in all we cannot decide between structures *a* and *b* Of course we might permit ourselves to proceed specula

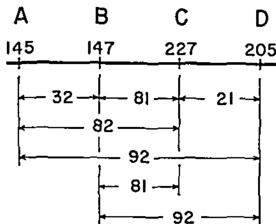


Fig 226 Portion of the map of the rII region of phage T4 (Doermann Chase and Edgar)

tively and see which structure lends itself better to the construction of mechanical models aimed at describing mating heterozygote formation and recombination However this approach has not been successful in narrowing down our choice

We will drop the heterozygote story momentarily at this point but will bear in mind its principal inference that the mating act produces in the first instance a vegetative phage particle involving a short overlap region *Thus we* cannot speak of the point on the genetic map which is involved in a recombination act In*stead we must speak of 2 points the boundaries* of the overlap region which belong to 1 recombination act If the 2 parental types happen to be genetically different within this overlap region we wind up with a phage heterozygote If the *parents* happen not to be different within the overlap region but outside of it then we wind up with a recombinant giving no evidence of its overlap region

Let us turn now to the more recent studies of genetic fine structure initiated by *Benzer* who devised a special and very powerful technic for the study of genetic recombination in those *cases* where the recombination frequency is very low The essence of this technic consists in the use of an indicator strain which does not propagate either of the types but is fully sensitive to 1 of the 2 reciprocal recombinants Plating on such an indicator strain the progeny of a cross permits one to detect and to count accurately 1 of the recombinants in the presence of an enormous excess of parental typ s The work of Benzer and that of the Doermann group which *we will describe presently* concerns one particular short region of the genetic map of phage T4 However the same technic can be and has been applied to other *regions*

The work of Benzer concerns the rII region of phage T4 The letter r designates a class of mutants producing large plaques on the usual host of this phage this host being strain *B* of *E coli* Benzer isolated over a thousand of these *r* mutants and discovered that a large proportion of them have the property in common that another strain of *E coli* strain K is not lysed by them These mutants infect the bacterium but shortly after that the processes leading to phage multiplication come to a standstill The r mutants which do not form plaques on K constitute the class rII They are by no means all identical On the contrary if two of them are crossed the chances are that they will form recombinants with a frequency ranging from 01 per cent to about 6 per cent

Benzer has made a very elaborate study of these mutants and the first step of this study consists in mapping It turns out also that in this case the linear mapping can be carried out consistently as *illustrated in Figure 226 An* exception is constituted by a small class of rII mutants which behave with respect to recom*bination as if they covered* an appreciable section of the rII region These do not form recombinants with any rII mutants in the covered section These sectional mutants have also an other property in common They do not back *mutate to the standard type an indication* that the mutational change has been more severe than in the case of all the simply mappable mutants Among these latter there are subgroups which seem to be strictly isolocal They form no *recombinants with each other* and this no really means no The resolving power of the method for the detection of recombinants is so great that it can detect them when they occur a hundred times more rarely than the 01 per cent actually observed as the smallest finite value

Benzer has found that the rII region actually consists of 2 contiguous portions *A* and *B* which control physiologically separate functions

If a bacterium K is infected with two rII mutants then the infection proceeds normally if one is an rIIA mutant and the other an rIIB mutant but it is abortive if both belong to the rIIA class or if both belong to the rIIB class This is a very important discovery in itself and it will be worth our while to linger over it for a moment The experiments demonstrated in the first place that among the r mutants there exists a subgroup with the physiologic peculiarity of not being able to multiply on strain K The mapping experiments then showed that this group of mutants also belongs together on the genetic map They recombine with a maximum frequency of 6 per cent Further they can be arranged among themselves on a linear map

Next on the physiologic side again this group of mutants splits up into 2 subgroups such that members of the same subgroup cannot cooperate to bring about phage growth in strain K while any member of subgroup A can cooperate with any member of subgroup P Next again on the genetic side it is found that the members of subgroup A occupy one segment of the genetic map while the members of subgroup B occupy a different segment which does not overlap with the first one but lies next to it What this shows very clearly is that the genetic information is organized into functional units strictly 1 dimensional as far as genetic analysis can tell and that these functional units are arranged in tandem on the map as a whole

Of course this is shown here only for two particular functional units and by no means should we jump to the conclusion that the whole of the genetic map is arranged as one long tape It is entirely conceivable and to my mind even probable that for longer distances another organizational principle may supervene just as long manuscripts are not published as tapes but *are broken up into lines and pages and volumes*

We mentioned before that Benzer found for his rII mutants that there exists a finite minimum recombination value about 01 per cent A similar value was found for a different region of the genetic map by Streisinger and Franklin The meaning of this minimum finite value is at present entirely a matter of speculation and shall not concern us here However we will follow Benzer in his analysis one step further in the study of those sub-subgroups of mutants which show zero recombination each with each The members of such a group are then genetically speaking strictly isolocal Benzer studied particularly one such group comprising 123 members The motive for undertaking such a study is that of wishing to find out something about the limits of variety that such a group of isolocal mutants can exhibit In other words how many shapes can one point on the genetic map have? Such a question of course comes quite naturally these days when we constantly have before our minds the picture of the DNA molecule as the physical bearer and repository of genetic information

Now a point on this molecule is certainly not less though possibly more than 1 nucleotide pair If it is just 1 nucleotide pair then presumably it cannot take more than 4 shapes corresponding to the 4 base pairs and thus our group of isolocal mutants should not include more than 3 varieties the fourth being the unmutated variety Among the 127 members of 1 isolocal group mentioned above Benzer did in fact find 3 varieties and not more distinguishable by their rate of reversion to the wild type So this looks very hopeful for the point of view which assumes that a point mutation is an exceedingly simple and narrowly circumscribed chemical change in a molecule whose structure is well understood A detailed study of mutagenesis in phage with mutations produced by 5 bromouracil using Benzer s criteria for identification of the mutant should enable us to tell a great deal more

With these ideas about genetic fine structure in mind let us now return to the problem of the mechanism of genetic recombination and of the role of the heterozygote in this mechanism To begin with let us consider a cross between 2 r mutants belonging to the same or to different physiological subunits of the rII region *What are we actually measuring when we make* such a cross and plate the progeny on the indicator strain K? In the first instance we will be counting to be sure true recombinants which are wild type with respect to both of these r locations But what about the heterozygotes? We had arrived previously at the notion that the recombinational event produces in the first instance a particle containing an overlap region of appreciable length much longer than the minimum recombinational distance Therefore

most of the primary products of recombinational events will be doubly heterozygous and some of them will be singly heterozygous Will these heterozygotes register on *K*? Fortunately we know the answer to this question They will register on K at least with appreciable probability if the 2 r mutants belong to different subunits but will not register when they belong to the same subunit The fact that the heterozygote does register on K when the two r mutants belong to different functional subunits means that these heterozygotes simulate the 2 recombinants and that they have to be corrected for when one wants to determine the recombination frequency This was not appreciated at first and made it appear as if there were a gap between regions A and B

The fact that the heterozygotes will not register when the markers belong to the same subunit tells us something new about the heterozygote It tells us that the functional unit fails to function not only when part of it is in one phage and part in the other phage but also if part of it is in one of the overlapping segments of the heterozygote and the other in its counterpart That these heterozygotes which fail to register on K are potent producers of true recombinants can be shown very elegantly If the original progeny of the cross is adsorbed on strain *B* before plating on strain K the plaque count increases and this increase is due to the fact that most of the heterozygotes whose overlap region covers both loci now do register They do so because they can function on *B* and during their 1 cycle of growth they need produce only 1 wild type recombinant to be able to continue to grow on K and to go on to produce a plaque They do produce a wild type recombinant much more often than if the bacterium had simply been infected with one each of the parental types This experiment shows perhaps more clearly than any of the others the pivotal role which the heterozygotes play for the recombination of closely linked markers

For such pairs recombination occurs largely in 2 steps In the first step a heterozygote is formed during a mating of the parental particles and the overlap region of this heterozygote covers for very close markers mostly both loci In the second step a true recombinant is formed

However at this point we are still in the dark We do not know what a heterozygote does when it replicates Does it copy itself as heterozygote or does it produce only segregants i e non heterozygous progeny? If it does produce only segregants does it produce one at a time through several replications so that it can throw off several segregants and perhaps even several kinds of segregants? Or does it produce 2 segregants at a time and perish during the first replication act? These questions have not yet been answered with any degree of certainty but presumably will be answered soon because new technics permit one to screen out the double heterozygotes and to study their progeny by single burst technics

Two rather qualitative things about the heterozygotes can be said

1 A single factor heterozygote produces principally parental types and not copies of itself

2 A double heterozygote involving 2 closely linked markers produces in addition to the parental types also recombinants and it produces these recombinants more frequently than a bacterium mixedly infected with the parental types However we do not know whether this excess of recombinants is due to a direct production during replication of the heterozygote or to a more indirect mechanism as follows The heterozygote first produces parental type offspring without however perishing in the act The recombinants are then produced during subsequent matings between the heterozygote and its offspring

Still other mechanisms involving the production of partial replicas and their transfer from one matrix to another have been discussed by Barricelli and by Bresch

Intimately connected with this 2 step nature of the formation of recombinants between closely linked markers is the phenomenon of negative interference studied intensively by Doermann Chase and Edgar The term interference is taken over from ordinary genetics in higher organisms There it signifies the fact that recombination in one region interferes with the occurrence of recombination in an adjacent region This interference is positive i e recombination in one region reduces the probability in an adjacent region In phage genetics we find the opposite—negative interference i e recombi

nation in one region increases the probability in an adjacent region This was first discovered for phage recombinations involving fairly distant markers and was explained very simply and satisfactorily by taking into account that a phage cross involves several rounds of mating and that the particles in the progeny have not all mated with their opposite genetic types equally often

Therefore by looking in the progeny at the class of particles which show recombination in one region we introduce a statistical bias in favor of those particles which have mated with the opposite genetic type at least once It is therefore reasonable that we should find in this selected class more recombinants with respect to another linked region than in the total progeny However Doermann and Chase discovered that there exists a much stronger negative interference than can be accounted for by this argument when short regions are involved In these studies the mutants of the rII region were again used By special technics Doermann and Chase succeeded in isolating from crosses between 2 r mutants not only the wild type recombinants but also the reciprocal recombinants carrying both the parental r markers Using these double r stocks as parental phage a great variety of 3 or 4 factor crosses could be carried out Let us symbolize the various r markers as a b c the alphabetical order corresponding to their order on the genetic map Numerous crosses of the following types were performed

(1) $\frac{ab+}{++c}$ (2) $\frac{a+c}{+b}$ (3) $\frac{a++d}{+bc+}$ (4) $\frac{a+c+}{+b+d}$

To produce a wild type particle the first cross requires recombination between b and c and no recombination between a and b The second cross requires 2 recombinations between a and b and between b and c The third cross requires recombination between a and b and between c and d and no recombination between b and c The fourth cross requires recombinations to occur in each of the 3 regions The experiments of Doermann and Chase show very convincingly in all of these cases that the recombinational events in these short regions are correlated and that this correlation effect is limited to regions of the length of the heterozygote overlap region In view of what we have said (p 350) about the 2 step nature of the production of recombinants when closely linked markers are involved this finding is eminently reasonable in the present case when we select in the progeny a particle which is recombinant in one region we select not only one which must have mated with an opposite partner but also one which very probably in its ancestry must have had one member which was heterozygous for most of the region considered Of course this introduces a strong bias in favor of other recombinations with the region Perhaps it will be possible from the detailed quantitative studies to arrive at definite conclusions about the behavior of heterozygotes during subsequent replications

The question whether reciprocal recombinants are formed pari passu or statistically independently must now be put separately for close in contrast with distant markers For distant markers where the overlap region is not likely to cover a marker the question stands much as before with the additional specification that the reciprocal recombinants would have to contain reciprocal overlaps The most detailed study is due to Bresch working with mutants of phage T1 The rationale of his experimental setup is the following We know that in 2 factor crosses reciprocal recombinants are formed statistically in equal numbers when the yield is averaged over a large number of single bursts However when one looks at individual single bursts one finds practically no correlation between the numbers of reciprocal recombinants produced This lack of correlation might mean that the reciprocal recombinants are formed independently of each other or it might mean that they are produced pairwise in the first instance but that this strict correlation is not apparent in the single bursts because it is wiped out by two randomizing influences affecting the two recombinants independently One of these randomizing influences is maturation the other is replication after recombination The latter influence can be reduced by disregarding the actual numbers of recombinants found in single bursts and scoring instead for presence or absence of a given recombinant Such a correlation for the presence of reciprocal recombinants should be the more striking the rarer the recombinant i e the closer the linkage between the markers However it is not possible for this experiment to take 2 markers belonging to the same func

tional unit because then only 1 of the reciprocal recombinants could be scored conveniently De tection of the other the double mutant would require elaborate backcrosses Thus close but not too close markers are needed

Another precaution that must be used is that of avoiding a confusion of a correlation due to the suspected pair production of the reciprocal recombinants with a spurious correlation due to other causes Principal among these spurious causes would be an inhomogeneity among a group of infected bacteria with respect to the total amount of mating taking place in each If there are some in which much mating takes place and some in which little mating takes place this also would lead to a correlated appear ance of reciprocal recombinants even if they are not produced in the same act

In Bresch s studies this spurious correlation was corrected for by making 3 factor crosses instead of 2 factor crosses In a 3 factor cross there are 6 recombinants and one can study the correlation not only between reciprocal pairs of recombinants but also between other pairs which are not reciprocal and therefore would not be expected to be made in one act Indeed Bresch found a weak correlation between reciprocal recombinants but exactly the same correlation was found between nonreciprocal pairs and both correlations were ascribed therefore to spurious causes Bresch s evidence then strongly favors statistical independence in the produc tion of reciprocal recombinants

However for close markers the situation is different Here as we have said the recom binants are formed in 2 steps The first step is *often the formation of the double heterozygote* but we must now focus our attention on the second step the formation of recombinants from *the heterozygote Are these produced singly or* as reciprocal pairs? To my knowledge this question has not yet been attacked experimen tally but very probably will be settled in the near future

Let us try to sum up to see where we stand Genetic mapping has disclosed the existence of linkage groups within linkage groups a gross linear order and within short regions again a linear order closely correlated with the physio logic functions controlled by the genetic mate rial Further a minimum recombination fre quency has been found and strictly isolocal mutants have been found to display only a small number of varieties These findings are compatible and in fact are in striking agree ment with expectations if it is assumed that DNA molecules constitute the physical counter part of the genetic information However they do not demand that the entire map is to be thought of as one long DNA chain

The picture of a phage cross as a series of matings in a small population of vegetative par ticles has been sustained A major novelty is the conclusion that the recombinational act involves not a point on the genetic map but a finite re gion The primary product is one particle con taining an overlap region not a pair of recipro cal recombinants In the overlap region both parental particles are equally represented If the parental particles differ in this region a hetero zygote results Outside the overlap region the particles are genetically pure on one side of the overlap representing one parent on the other side the other parent The physical nature of this overlap is uncertain Specifically it is un certain whether the particle is *thicker in the* overlap region than outside For recombinations between closely linked markers the overlap fea ture becomes very important A large propor tion of the recombinants are formed in two successive steps the first leading to the forma tion of an overlap longer than the distance between a close pair of markers the second leading to the formation of the recombinant proper The details of the behavior of the overlap region during subsequent replication and mating are still very much in dispute

BIPLIOGRAPHY

Adams M H Criteria for a biological classifi cation of bacterial viruses *Ann New York Acad Sc* 56 442 1953

—— The hybridization of coliphage T5 and salmonella phage PB J Immunol 67 313 1951

Baylor M B Hurst D D Allen S L and Berfani E T The frequency and distribu tion of loci affecting host range in phage T? 1957

Benzer S The elementary units of heredity *in* McElroy and Glass (eds) The Chemical Basis of Heredity Baltimore Johns Hopkins Press 1957

——— Fine structure of a genetic region in bacteriophage Proc Nat Acad Sc 41 344 1955
Bresch C Zum Paarungsmechanismus von Bakteriophagen Ztschr Naturforschung 10b 545 1955
Bresch and Menningmann Weitere Untersuchungen zur Genetik von T1 Phagen Ztschr Naturforschung 9b 212 1954
Bresch C and Trautner T Die Bedeutung des Zweifarb-Naehrbodens fuer genetische Untersuchungen am Phagen T1 Ztschr Ind Abst u Vererbungslehre 87 590 1956
——— Zur Kinetik der Rekombinantenbildung bei T1 Ztschr Naturforschung 10b 436 1955
Cohen S S The biosynthesis of nucleic acids in some microbial systems *in* McElroy and Glass (eds) The Chemical Basis of Heredity Baltimore Johns Hopkins Press 1957
Delbruck M and Stent G S On the mechanism of DNA replication *in* McElroy and Glass (eds) The Chemical Basis of Heredity Baltimore Johns Hopkins Press 1957
Doermann A M The vegetative state in the life cycle of bacteriophage etc Cold Spring Harbor Symp Quant Biol 18 3 1953
Doermann A M Chase M and Edgar R Personal communication
Doermann A M and Hill P Genetic structure of phage T4 etc Genetics 38 79 1953
Edgar R Personal communication
Hershey A D and Chase M Genetic recombination and heterozygosis in phage Cold Spring Harbor Symp Quant Biol 16 471 1951
Hershey H D and Rotman R Genetic recombination between host range and plaque type mutants of phage in single bacterial cells Genetics 34 44 1949
Jacob F and Wollman E L Le système genetique du bacteriophage Ann Inst Pasteur 87 653 1954 Effet du UV sur le recombinaison génetique Ann Inst Pasteur 88 724 1955
Jesaitis M A Differences in the chemical composition of the phage nucleic acids Nature 178 637 1956
Kaiser A D A genetic study of the temperate coliphage lambda Virology 1 424 1955
Levinthal C Recombination in phage T2 its relationship to heterozygosis and growth Genetics 39 169 1954
Litman R M and Pardee A B Production of phage mutants by a disturbance of DNA metabolism Nature 178 529 1956
Murphy J S Recombinations of mutant phages of Bacillus megatherium 899 J Exper Med 98 657 1953
Sansome E Crossing over in fungi bacteria bacteriophage and higher organisms C R Carlsberg Lab Ser Physiol 26 315 1956
Sinsheimer R L Glucose content of the DNA of certain phages Proc Nat Acad Sc 42 507 1956
Stent G S Mating in the reproduction of bacterial viruses Advances Virus Res 5 95 1958
Streisinger G Phenotypic mixing of host range and serological specificities in phages T2 and T4 Virology 2 388 1956
Streisinger G and Franklin N C Mutation and recombination at the host range genetic region of phage T2 Cold Spring Harbor Symp Quant Biol 21 103 1957
Streisinger G and Weigle J Properties of phages T2 and T4 with unusual inheritance Proc Nat Acad Sc 42 504 1956
Tomizawa J and Sunakawa S Effect of chloramphenicol on DNA synthesis and the development of UV resistance in *E coli* infected with T2 J Gen Physiol 39 553 1956
Visconti N and Delbruck M The mechanism of genetic recombination in phage Genetics 38 5 1953
Volkin E and Astrachan Intracellular distribution of labeled RNA after phage infection of *E coli* Virology 2 433 1956
——— Phosphorus incorporation in *E coli* RNA after infection with phage T2 Virology 2 149 1956
——— RNA metabolism in T2 infected *E coli* *in* McElroy and Glass (eds) The Chemical Basis of Heredity Baltimore Johns Hopkins Press 1957
Weigle J J Induction of mutations in a bacterial virus Proc Nat Acad Sc 39 628 1953
Wollman E L and Jacob F Mecanism de la recombinaison genetique Ann Inst Pasteur 87 674 1954

Viral Latency

DR KENNETH M SMITH

Here is a definition of latency as suggested to me by Dr Robley Williams Latency is a nonspecific term indicative of our ignorance of what *is transpiring*

INTRODUCTION

There is much confusion of thought regarding viral latency and opinions differ as to whether it consists of a special kind of virus or a special kind of host Luria considers that viral latency is not a special situation but only an extreme one and that any virus must have some host in which a balance has been achieved whereby the continuation of the virus is ensured Partial latency and by that presumably is meant latency in some hosts is almost a necessity for every virus

I define viral latency broadly as the condition in which an organism is infected with a virus but shows no external evidence of the fact That the virus is present means that the infected cells are internally different from uninfected cells As seen from examples given later this fact is sometimes demonstrable directly by current techniques but in some examples of latency it is not

The course of events leading to the condition of latency can be divided arbitrarily into 4 types First there are viruses that cause evident symptoms when they first infect an organism these symptoms disappear but may recur at intervals as the consequence of some stimulus An example of this is herpes simplex in man

Second there are viruses that also initially cause a disease but the host recovers and although it still contains the virus never again shows external evidence of doing so Belonging to this category perhaps is the virus of lymphocytic choriomeningitis in mice referred to by Andrewes as an indigenous virus Traub showed that the virus persisted in the blood of mice after recovery from infection and that female mice thus carrying virus could infect their young in the uterus After the passage of 2 years the virus in the stock mice had become milder and milder so that its presence could be demonstrated only by inoculation into a non carrying strain of mice This is a rare case in *the field of animal viruses of the use of an* indicator host However in plant virus work the use of such indicators is one of the routine methods of detecting the presence of latent viruses *Several plant viruses can be put in this* second category where an initial reaction is caused notably the tomato black ring virus and other viruses of the ringspot type The criticism may be made that viruses of this second category are not strictly speaking latent because the host has gained the upper hand insofar as the virus persists but may not be able to multiply sufficiently to cause observable symptoms However the fact that the virus persists and that symptoms are absent are sufficient to warrant inclusion under our definition of latency

To the third category belong those viruses which apparently never cause a disease in their original host and cannot so far as I am aware be induced to do so Of course the original host in this context is the host in which the virus was originally discovered In this group are mainly plant viruses but the salivary gland virus of guinea pigs which does not cause a disease in the original individual may provide a *comparable type of phenomenon in animals* Three plant viruses which seem to be truly *latent in their original host are the paracrinkle* virus of the King Edward VII potato the latent virus of dodder *Cuscuta sp* and a virus which occurs commonly in sugar beets and mangolds

The fourth category may represent a fundamentally different condition the original hosts are not known ever to have shown symptoms and they seem to be free from detectable virus until they are subjected to some appropriate stimulus when a virus immediately becomes detectable and usually kills the host Examples of this type occur in insects and in the *lysogenic* bacteria This is referred to again later

There is some controversy as to whether

latency can be differentiated from masking or whether such a thing as masking exists at all Shope considers that the rabbit papilloma virus is masked in domestic rabbits and its presence can be demonstrated only with difficulty and by indirect means However Beard disputes this and says that masking in tumor viruses can be explained on the basis of low titer alone However this is not the case with the lysogenic bacteria where the phage appears to be in a different physical state Some of the latent insect viruses also possibly may come under the heading of masking rather than of latency

To summarize The presence of a latent virus may be shown in 1 of 3 ways either the host develops the disease spontaneously after a passage of time or the disease can be induced by various methods of treatment or in order to see the virus at all it must be transferred to a new host

SOME EXAMPLES OF LATENT VIRUSES

Plant Viruses

With the possible exception of the insects more viruses apparently are latent in plants than in any other type of organism Indeed it is probable that only a few of the viruses latent in weeds and other wild plants have been discovered yet

Latent viruses have been detected in hops strawberries potatoes raspberries dahlias weeds of various sorts sugar beets and even in giant trees of the tropical forests It is becoming increasingly clear that there is a group of soil borne viruses that cause ringspot symptoms in some hosts and occur in a range of apparently normal weeds and other plants When some of these viruses infect cultivated crops such as raspberries lettuce and tomatoes they cause quite severe diseases and become of economic importance

One of the most interesting of these latent plant viruses is that from the sugar beets previously mentioned as being in the third category of latent viruses This virus usually can be found in the commercial sugar beets in the British Isles and other countries notably the U S A Although it occurs commonly in sugar beets all attempts to find how it spreads have failed and tests for insect vectors and for soil and seed transmission have proved to be negative

Insect Viruses

Latent viruses occur in abundance in the larval forms of insects mainly the Lepidoptera These are of the polyhedral type and both the nuclear and the cytoplasmic kinds occur in the latent condition There seems to be little doubt that large populations of insects exist in which the greater proportion carry a latent virus This is particularly true of the garden tiger moth *Arctia caja* of the silkworm *Bombyx mori* and of the spruce budworm (*Choristoneura fumiferana* [Clem]) in Canada

Viruses in Higher Animals

To mention a few of the many latent viruses which have been discovered in higher animals rabbits carry Virus III in the testes mice have Theiler s encephalitis virus and the virus of lymphocytic choriomeningitis previously mentioned Carr says it cannot be emphasized too strongly that the majority of commercial stocks of fowls are naturally carriers of the viruses from an early age of the leukosis complex to which group the sarcoma and the leukemia viruses belong Fowls are also capable of carrying the virus of fowlpox

DETECTION OF LATENT VIRUSES

In Plants

1 By Means of Indicator Plants This method is dependent on finding a susceptible plant host which will react in an unmistakable manner to the virus latent in the plant under test The discovery of such a plant is a matter of trial and error although certain plant species do react clearly to an unusually large number of viruses These include the French bean *Phaseolus vulgaris* the cowpea *Vigna sinensis* and certain chenopodiaceous plants like *Chenopodium amaranticolor* and *Gomphrena globosa* For example inoculation of the sap from sugar beets containing the latent virus previously mentioned produces on the inoculated leaves of cowpea characteristic local lesions within 4 days The tobacco plant *Nicotiana tabacum* is susceptible to infection with more viruses than any other known plant and so makes another useful indicator host

2 By Serology Many plant viruses are excellent antigens and if partially purified and inoculated into a suitable animal such as the rabbit each gives rise to an antiserum which reacts only with that virus and related strains This is a useful tool in plant virus studies and has a special application to the investigation of latent viruses First it affords a rapid and delicate test for the presence of a latent virus and second it may reveal unsuspected relationships between latent viruses The technic has been used recently by Kassanis in an investigation of 3 viruses which may occur latent in potato and carnation plants By this means he has shown that a relationship exists between a latent virus which is insect borne and 2 which are not and thus has thrown light on a problem which has long been obscure This matter is further discussed in the section headed Mutation and Latency in Plant Viruses

Some of the more progressive seed potato growers now use the serologic test to detect the presence of the latent potato virus X so that they can select for propagation plants that are virus free This virus is widespread in some potato varieties and even though it causes no observable symptoms it can still reduce the yield by as much as 10 per cent

3 By Electron Microscopy This method is still only of academic interest since the labor of searching for a latent virus in a plant cell is considerable unless the virus is known to be of a characteristic and easily recognizable shape It has perhaps a limited application in the cases of rod shaped or filamentous viruses such as that of a latent tobacco mosaic or potato virus X but these are detected more easily by other means

In Insects

Latent viruses affecting the larval stages of insects are perhaps most easily detected by various methods of stimulation and this subject is discussed at greater length in the Section headed Stimulation of Latent Viruses

Not much work has been done on the electron microscopy of latent insect viruses but there is one case on record of what possibly might be a latent virus revealed by this method In the blood of apparently normal house crickets (*Gryllulus domesticus*) masses of viruslike particles were observed

In Higher Animals

Various methods are available for detecting the presence of latent viruses in the higher animals The salivary gland virus of guinea pigs was detected first by means of intracellular inclusions observed on the optical microscope

Stimulation by different means is another approach to the problem while the most efficient method is probably by serologic reactions

STIMULATION OF LATENT VIRUSES

By Serial Passage

Serial inoculation in animals sometimes leads to the development of symptoms presumably because the latent virus has increased in virulence or has been stimulated to multiply more vigorously For example two latent viruses in mice can be brought to light by this method Progressive inoculation with lung extracts frequently produces typical pneumonia from which a pneumonia virus of mice can be isolated Similar serial transmission stimulates into activity Theiler s mouse encephalitis virus

Little information is found on the effect of the inoculation of body fluids in progressive series through insects though it is probable that a latent virus might easily be activated by this procedure

Neither is there precise information on the effect of serial passage on latent plant viruses but presumably a tolerant host would continue to be a tolerant host after serial passage through the sames plant species In this connection serial passage of some viruses not necessarily latent through unusual plant hosts produces an apparent change of virulence which usually is due to a selection of one virus strain from a mixture of strains Possibly during serial passage of a latent plant virus a second latent virus could be picked up on the way when the combination might produce observable symptoms There are instances in some plants potatoes for example of more than one latent virus existing together

By the Use of Artificial Irritants and Chemicals

Many latent viruses can be stirred readily into activity by artificial stimulations of different kinds A carrier of the Rous I fowl sarcoma reacts violently to injection with methylcholanthrene resulting in the formation of a Rous I

sarcoma In the Shope papilloma virus in both domestic and cottontail rabbits applications of tar cause the papillomas to become malignant Here presumably the action is in changing the response of the cell to the virus Similarly in fowls carrying the virus of fowlpox the virus can be awakened by painting with hydrocarbons Here again the disease progresses much beyond the normal until a malignant growth develops but identical experiments carried out on fowls free of the virus failed to have any effect

Andrewes points out that the virus of mouse hepatitis apparently exists as a purely latent infection of many stocks of mice However unlike some latent mouse viruses serial transmission does not stimulate the hepatitis virus into action The equilibrium can be upset by the injection of another parasite *Eperythrozoon coccoides* This appears to activate the virus in some way which becomes evident after further serial passage

The work of Yamafuji has shown that apparently normal silkworms (*Bombyx mori*) develop polyhedral virus disease after feeding on foliage contaminated with some chemicals such as nitrites Yamafuji s interpretation of this is that the virus is produced de novo but in view of the very high incidence of latent polyhedral viruses in lepidopterous caterpillars the alternative hypothesis of stimulation of a latent virus seems to be preferable

The lysogenic bacteria represent a different situation from that of most latent viruses The virus seems not to exist as particles such as occur in vitro for infectious phage cannot be liberated by the mechanical breaking open of the lysogenic bacteria Its state is indicated by the name prophage and it must undergo some kind of maturation process before it becomes virulent However it is possible to induce mass lysis and the liberation of infectious phage by certain kinds of artificial stimulation notably ultraviolet light and treatment with hydrogen peroxide

By Inoculation with a Foreign Virus

Some good examples of virus stimulation by inoculation with a foreign virus have been found in insects To understand this it is necessary to mention briefly the different types of insect viruses First there are the polyhedroses in which certain tissues become filled with virus containing polyhedral crystals (there are 2 types of polyhedral diseases the nuclear and the cytoplasmic in which the viruses multiply respectively in cell nucleus or cytoplasm) second there are the granuloses in which the tissues become filled with minute granules containing the virus usually a single rod shaped particle per granule and third there are the diseases in which the virus is free in the tissues without intracellular inclusions

Preliminary cross inoculation studies have shown that inoculation with a nuclear polyhedral virus frequently will stimulate into action a latent cytoplasmic virus This may have practical significance There is an effort being made to use viruses in the control of certain insect pests We have been experimenting with a certain rather virulent pest but we could not find the virus So we gave it a dose of a virus of a different kind from the larvae of a butterfly and in one experiment it produced 100 per cent mortality but with an entirely different virus In other words the nuclear virus which we fed to the caterpillar invoked a latent virus which apparently did not occur in nature By that I mean that we have never been able to find in the insect the naturally occurring virus diseases of its kind but having once stimulated the cytoplasmic virus we could then pass it on indefinitely in series Search for a naturally occurring virus in the larva of the winter moth *Operophtera brumata* has failed but inoculation of apparently healthy winter moth larvae with a foreign nuclear polyhedral virus from the larvae of the painted lady butterfly *Vanessa cardui* produced a high mortality Examination of the dead winter moth larvae showed that they had died from a cytoplasmic polyhedrosis Once stimulated this virus was indefinitely transmissible in series At the moment observations appear to be lacking as to whether or not the reverse phenomenon is true i.e whether or not inoculation with a cytoplasmic polyhedral virus will stimulate a latent nuclear polyhedrosis Apparently a granulosis virus cannot stimulate a latent polyhedral virus or vice versa

In higher animals the virus of herpes simplex in man is frequently stimulated into action by the presence of the common cold virus

When the papilloma virus from the cottontail rabbit is inoculated into domestic rabbits a papil

2 **By Serology** Many plant viruses are excellent antigens and if partially purified and inoculated into a suitable animal such as the rabbit each gives rise to an antiserum which reacts only with that virus and related strains This is a useful tool in plant virus studies and has a special application to the investigation of latent viruses First it affords a rapid and delicate test for the presence of a latent virus and second it may reveal unsuspected relationships between latent viruses The technic has been used recently by Kassanis in an investigation of 3 viruses which may occur latent in potato and carnation plants By this means he has shown that a relationship exists between a latent virus which is insect borne and 2 which are not and thus has thrown light on a problem which has long been obscure This matter is further discussed in the section headed Mutation and Latency in Plant Viruses

Some of the more progressive seed potato growers now use the serologic test to detect the presence of the latent potato virus X so that they can select for propagation plants that are virus free This virus is widespread in some potato varieties and even though it causes no observable symptoms it can still reduce the yield by as much as 10 per cent

3 **By Electron Microscopy** This method is still only of academic interest since the labor of searching for a latent virus in a plant cell is considerable unless the virus is known to be of a characteristic and easily recognizable shape It has perhaps a limited application in the cases of rod shaped or filamentous viruses such as that of a latent tobacco mosaic or potato virus X but these are detected more easily by other means

In Insects

Latent viruses affecting the larval stages of insects are perhaps most easily detected by various methods of stimulation and this subject is discussed at greater length in the Section headed Stimulation of Latent Viruses

Not much work has been done on the electron microscopy of latent insect viruses but there is one case on record of what possibly might be a latent virus revealed by this method In the blood of apparently normal house crickets (*Gryllulus domesticus*) masses of viruslike particles were observed

In Higher Animals

Various methods are available for detecting the presence of latent viruses in the higher animals The salivary gland virus of guinea pigs was detected first by means of intracellular inclusions observed on the optical microscope

Stimulation by different means is another approach to the problem while the most efficient method is probably by serologic reactions

STIMULATION OF LATENT VIRUSES

By Serial Passage

Serial inoculation in animals sometimes leads to the development of symptoms presumably because the latent virus has increased in virulence or has been stimulated to multiply more vigorously For example two latent viruses in mice can be brought to light by this method Progressive inoculation with lung extracts frequently produces typical pneumonia from which a pneumonia virus of mice can be isolated Similar serial transmission stimulates into activity Theiler s mouse encephalitis virus

Little information is found on the effect of the inoculation of body fluids in progressive series through insects though it is probable that a latent virus might easily be activated by this procedure

Neither is there precise information on the effect of serial passage on latent plant viruses but presumably a tolerant host would continue to be a tolerant host after serial passage through the sames plant species In this connection serial passage of some viruses not necessarily latent through unusual plant hosts produces an apparent change of virulence which usually is due to a selection of one virus strain from a mixture of strains Possibly during serial passage of a latent plant virus a second latent virus could be picked up on the way when the combination might produce observable symptoms There are instances in some plants potatoes for example of more than one latent virus existing together

By the Use of Artificial Irritants and Chemicals

Many latent viruses can be stirred readily into activity by artificial stimulations of different kinds A carrier of the Rous I fowl sarcoma reacts violently to injection with methylcholanthrene re ulting in the formation of a Rous I

sarcoma In the Shope papilloma virus in both domestic and cottontail rabbits applications of tar cause the papillomas to become malignant Here presumably the action is in changing the response of the cell to the virus Similarly in fowls carrying the virus of fowlpox the virus can be awakened by painting with hydrocarbons Here again the disease progresses much beyond the normal until a malignant growth develops but identical experiments carried out on fowls free of the virus failed to have any effect

Andrewes points out that the virus of mouse hepatitis apparently exists as a purely latent infection of many stocks of mice However unlike some latent mouse viruses serial transmission does not stimulate the hepatitis virus into action The equilibrium can be upset by the injection of another parasite *Eperythrozoon coccoides* This appears to activate the virus in some way which becomes evident after further serial passage

The work of Yamafuji has shown that apparently normal silkworms (*Bombyx mori*) develop polyhedral virus disease after feeding on foliage contaminated with some chemicals such as nitrites Yamafuji s interpretation of this is that the virus is produced de novo but in view of the very high incidence of latent polyhedral viruses in lepidopterous caterpillars the alternative hypothesis of stimulation of a latent virus seems to be preferable

The lysogenic bacteria represent a different situation from that of most latent viruses The virus seems not to exist as particles such as occur in vitro for infectious phage cannot be liberated by the mechanical breaking open of the lysogenic bacteria Its state is indicated by the name prophage and it must undergo some kind of maturation process before it becomes virulent However it is possible to induce mass lysis and the liberation of infectious phage by certain kinds of artificial stimulation notably ultraviolet light and treatment with hydrogen peroxide

By Inoculation with a Foreign Virus

Some good examples of virus stimulation by inoculation with a foreign virus have been found in insects To understand this it is necessary to mention briefly the different types of insect viruses First there are the polyhedroses in which certain tissues become filled with virus containing polyhedral crystals (there are 2 types of polyhedral diseases the nuclear and the cytoplasmic in which the viruses multiply respectively in cell nucleus or cytoplasm) second there are the granuloses in which the tissues become filled with minute granules containing the virus usually a single rod shaped particle per granule and third there are the diseases in which the virus is free in the tissues without intracellular inclusions

Preliminary cross inoculation studies have shown that inoculation with a nuclear polyhedral virus frequently will stimulate into action a latent cytoplasmic virus This may have practical significance There is an effort being made to use viruses in the control of certain insect pests We have been experimenting with a certain rather virulent pest but we could not find the virus So we gave it a dose of a virus of a different kind from the larvae of a butterfly and in one experiment it produced 100 per cent mortality but with an entirely different virus In other words the nuclear virus which we fed to the caterpillar invoked a latent virus which apparently did not occur in nature By that I mean that we have never been able to find in the insect the naturally occurring virus diseases of its kind but having once stimulated the cytoplasmic virus we could then pass it on indefinitely in series Search for a naturally occurring virus in the larva of the winter moth *Operophtera brumata* has failed but inoculation of apparently healthy winter moth larvae with a foreign nuclear polyhedral virus from the larvae of the painted lady butterfly *Vanessa cardui* produced a high mortality Examination of the dead winter moth larvae showed that they had died from a cytoplasmic polyhedrosis Once stimulated this virus was indefinitely transmissible in series At the moment observations appear to be lacking as to whether or not the reverse phenomenon is true i e whether or not inoculation with a cytoplasmic polyhedral virus will stimulate a latent nuclear polyhedrosis Apparently a granulosis virus cannot stimulate a latent polyhedral virus or vice versa

In higher animals the virus of herpes simplex in man is frequently stimulated into action by the presence of the common cold virus

When the papilloma virus from the cottontail rabbit is inoculated into domestic rabbits a papil

2 By Serology Many plant viruses are excellent antigens and if partially purified and inoculated into a suitable animal such as the rabbit each gives rise to an antiserum which reacts only with that virus and related strains This is a useful tool in plant virus studies and has a special application to the investigation of latent viruses First it affords a rapid and delicate test for the presence of a latent virus and *second it may reveal unsuspected relationships* between latent viruses The technic has been used recently by Kassanis in an investigation of 3 viruses which may occur latent in potato and carnation plants By this means he has shown that a relationship exists between a latent virus which is insect borne and 2 which are not and thus has thrown light on a problem which has long been obscure This matter is further discussed in the section headed Mutation and Latency in Plant Viruses

Some of the more progressive seed potato growers now use the serologic test to detect the presence of the latent potato virus X so that they can select for propagation plants that are virus free This virus is widespread in some potato varieties and even though it causes no observable symptoms it can still reduce the yield by as much as 10 per cent

3 By Electron Microscopy This method is still only of academic interest since the labor of searching for a latent virus in a plant cell is *considerable unless the virus is known to be of a* characteristic and easily recognizable shape It has perhaps a limited application in the cases of rod shaped or filamentous viruses such as that of a latent tobacco mosaic or potato virus X but these are detected more easily by other means

In Insects

Latent viruses affecting the larval stages of insects are perhaps most easily detected by various methods of stimulation and this subject is discussed *at greater length in the Section headed* Stimulation of Latent Viruses

Not much work has been done on the electron microscopy of latent insect viruses but there is one case on record of what possibly might be a latent virus revealed by this method In the blood of apparently normal house crickets (*Gryllulus domesticus*) masses of viruslike particles were observed

In Higher Animals

Various methods are available for detecting the presence of latent viruses in the higher animals The salivary gland virus of guinea pigs was detected first by means of intracellular inclusions observed on the optical microscope

Stimulation by different means is another approach to the problem while the most efficient method is probably by serologic reactions

STIMULATION OF LATENT VIRUSES

By Serial Passage

Serial inoculation in animals sometimes leads to the development of symptoms presumably because the latent virus has increased in virulence or has been stimulated to multiply more vigorously For example two latent viruses in mice can be brought to light by this method Progressive inoculation with lung extracts frequently produ es typical pneumonia from which a pneumonia virus of mice can be isolated Similar serial transmission stimulates into activity Theiler s mouse encephalitis virus

Little information is found on the effect of the inoculation of body fluids in progressive series through insects though it is probable that a latent virus might easily be activated by this procedure

Neither is there precise information on the effect of serial passage on latent plant viruses but presumably a tolerant host would continue to be a tolerant host after serial passage through the sames plant species In this connection serial passage of some viruses not necessarily la en through unusual plant hosts produces an apparent change of virulence which usually is due to *a selection of one virus strain from* a mixture of *strains* Possibly during serial passage of a latent plant virus a second latent virus could be picked up on the way when the combination might produce observable symptoms There are instances in some plants potatoes for example of more than one latent virus existing *together*

By the Use of Artificial Irritants and Chemicals

Many latent viruses can be stirred readily into activity by artificial stimulations of different kinds A carrier of the Rous I fowl sarcoma reacts violently to injection with methylcholanthrene resulting in the formation of a Rous I

sarcoma In the Shope papilloma virus in both domestic and cottontail rabbits applications of tar cause the papillomas to become malignant Here presumably the action is in changing the response of the cell to the virus Similarly in fowls carrying the virus of fowlpox the virus can be awakened by painting with hydrocarbons Here again the disease progresses much beyond the normal until a malignant growth develops but identical experiments carried out on fowls free of the virus failed to have any effect

Andrewes points out that the virus of mouse hepatitis apparently exists as a purely latent infection of many stocks of mice However unlike some latent mouse viruses serial transmission does not stimulate the hepatitis virus into action The equilibrium can be upset by the injection of another parasite *Eperythrozoon coccoides* This appears to activate the virus in some way which becomes evident after further serial passage

The work of Yamafuji has shown that apparently normal silkworms (*Bombyx mori*) develop polyhedral virus disease after feeding on foliage contaminated with some chemicals such as nitrites Yamafuji's interpretation of this is that the virus is produced de novo but in view of the very high incidence of latent polyhedral viruses in lepidopterous caterpillars the alternative hypothesis of stimulation of a latent virus seems to be preferable

The lysogenic bacteria represent a different situation from that of most latent viruses The virus seems not to exist as particles such as occur in vitro for infectious phage cannot be liberated by the mechanical breaking open of the lysogenic bacteria Its state is indicated by the name prophage and it must undergo some kind of maturation process before it becomes virulent However it is possible to induce mass lysis and the liberation of infectious phage by certain kinds of artificial stimulation notably ultraviolet light and treatment with hydrogen peroxide

By Inoculation with a Foreign Virus

Some good examples of virus stimulation by inoculation with a foreign virus have been found in insects To understand this it is necessary to mention briefly the different types of insect viruses First there are the polyhedroses in which certain tissues become filled with virus containing polyhedral crystals (there are 2 types of polyhedral diseases the nuclear and the cytoplasmic in which the viruses multiply respectively in cell nucleus or cytoplasm) second there are the granuloses in which the tissues become filled with minute granules containing the virus usually a single rod shaped particle per granule and third there are the diseases in which the virus is free in the tissues without intracellular inclusions

Preliminary cross inoculation studies have shown that inoculation with a nuclear polyhedral virus frequently will stimulate into action a latent cytoplasmic virus This may have practical significance There is an effort being made to use viruses in the control of certain insect pests We have been experimenting with a certain rather virulent pest but we could not find the virus So we gave it a dose of a virus of a different kind from the larvae of a butterfly and in one experiment it produced 100 per cent mortality but with an entirely different virus In other words the nuclear virus which we fed to the caterpillar invoked a latent virus which apparently did not occur in nature By that I mean that we have never been able to find in the insect the naturally occurring virus diseases of its kind but having once stimulated the cytoplasmic virus we could then pass it on indefinitely in series Search for a naturally occurring virus in the larva of the winter moth *Operophtera brumata* has failed but inoculation of apparently healthy winter moth larvae with a foreign nuclear polyhedral virus from the larvae of the painted lady butterfly *Vanessa cardui* produced a high mortality Examination of the dead winter moth larvae showed that they had died from a cytoplasmic polyhedrosis Once stimulated this virus was indefinitely transmissible in series At the moment, observations appear to be lacking as to whether or not the reverse phenomenon is true ie whether or not inoculation with a cytoplasmic polyhedral virus will stimulate a latent nuclear polyhedrosis Apparently a granulosis virus cannot stimulate a latent polyhedral virus or vice versa

In higher animals the virus of herpes simplex in man is frequently stimulated into action by the presence of the common cold virus

When the papilloma virus from the cottontail rabbit is inoculated into domestic rabbits a papil

loma is produced in which virus cannot be demonstrated However if the papilloma virus is mixed with the virus of sheep dermatitis and then inoculated to the domestic rabbit papillomas are formed from which active infective papilloma virus can be recovered

By Environmental Conditions

Adverse environmental conditions sometimes stimulate a latent virus into obvious activity For example parrots when kept together under dirty insanitary conditions frequently suffer an outbreak of psittacosis Also it is common for caterpillars of the puss moth (*Cerura vinula*) to develop a polyhedral virus disease under conditions of high humidity and low temperature

Environmental conditions such as temperature and light intensity have a great influence on the development of symptoms in plant virus diseases and on the rate of virus multiplication but there does not seem to be any information on the actual stimulation of a latent plant virus by environmental conditions

VIRUS MASKING

Beard considers that masking of symptoms follows simply on a too small virus concentration alone but Shope defines a masked virus as one which is known by circumstantial evidence or by a series of indirect tests to be present but is not of itself directly demonstrable One example of a masked animal virus is that of rabbit papilloma already briefly referred to This virus causes warts on the skin of cottontail rabbits in the Middle Western states of the U S A and is easily transmitted in series indefinitely in the cottontail When inoculated into the domestic rabbit similar warts are formed but virus is not transmissible from them either to cottontail or domestic rabbits However there are indirect means of proving that the papilloma virus is present in the domestic rabbit tumors These methods are immunologic and make use of the virus neutralization test or the demonstration of active immunity Luria points out that there is more evidence of the actual existence of virus possibly in modified form in domestic rabbit papillomas It is occasionally possible to demonstrate the virus in filtrates of extracts of these papillomas by injecting the filtrates into skin rendered hypoplastic by nonspecific irritants Moreover in 2 cases serial transmission in the domestic rabbit for 18 and 14 passages has been successful Return of the virus to the cottontail rabbit for one passage gave rise immediately to a strain of the cottontail type nontransferable to the domestic rabbit

Reference has been made in the previous section to the experiments of Selbie who was able to obtain active papilloma virus from domestic rabbit tumors if the sheep dermatitis virus was added We know little of the peculiar interreactions of mixed virus infections and somewhat similar phenomena occur in plant virus diseases Examples are known of 2 viruses occurring together naturally in one plant and while together in the plant both viruses are aphid borne However when separated 1 of the viruses loses its insect transmissibility

Shope gives another example of a masked animal virus this is the virus of swine influenza Here there is a reservoir host mechanism which keeps the virus going for some 9 months of the year between epidemics Virus cannot be detected by direct means either in the larval lung worms or in the intermediate host (earthworm) or in the adult longworm after transmission to the pig Swine which have become parasitized with lungworms known to be carriers of the masked virus do not come down directly with swine influenza They must have some provocative stimulus and the most successful consists of injections of the bacterium *H influenzae suis* Curiously enough this provocation of masked influenza virus succeeds only under experimental conditions between September and April

Temporary masking of the symptoms in virus diseased plants can be induced by environmental changes High temperatures are the most effective but low temperature masking is also known In the case of *Abutilon* mosaic the symptoms disappear when the plants are kept in the dark for a period

MUTATION AND LATENCY IN PLANT VIRUSES

The degree of mutation of which plant viruses are capable is probably much greater than has hitherto been supposed and this has given rise to some interesting problems The insect transmissibility of a particular plant virus is apparently not an immutable property but is liable to undergo considerable modification in which the host appears to play a part

Holmes isolated a strain of tobacco mosai which has changed its relationship to the tobacco plant so that it is completely symptomless in that host He called this a masked strain but since it is easily transmissible from the tobacco plant it is strictly speaking latent in the sense that observable symptoms are not produced A similar case was reported by Salaman for potato virus X in the tobacco plant

By far the most intriguing and puzzling example of plant virus latency is that of potato paracrinkle This virus was discovered first by Salaman and LePelley and it is latent without exception in every potato plant of the variety King Edward VII Moreover the virus has no natural means ot spread and has never been observed to occur naturally in any other host plant Therefore the question is how did the virus get into the original King Edward VII seedling in the first place Only recently has some light been thrown on this problem Kassanis has found that para rinkle virus is related serologically to 2 other latent viruses potato virus S and a carnation latent virus One of the 3 the carnation virus is aphid borne This suggests a feasible explanation for the para crinkle virus problem i e that it was carried in the first place by aphids from carnations to the original King Edward VII potato seedling and once there lost by mutation its inse t transmissibility Of course after that the virus would be passed continuously by vegetative propagation in the tubers

DISCUSSION

The main questions seem to be is a latent virus different from a nonlatent virus and is it changed in any way by the various techni s which make it evident? There is no good evidence that a latent virus is chemically or physically different from one which is nonlatent except in one or two possible instances referred to later

A plant virus which is latent in one host but is virulent in another does not differ so far as we know in any way when extracted from the respective host plants Most of the differences in severity of symptoms caused could be accounted for by different concentrations of virus and the effect of the various methods of stimulation would be merely one of increasing virus titer If this is the case it would be more correct to speak of a latent infection" rather than of a latent virus

However we must remember that viruses sometimes can be changed qualitatively by transmission to certain hosts and not only quantitatively For example we know that one strain of tobacco mosai virus apparently mutates on transmission between beans and tobacco and potato virus C varies in its insect transmissibility according to its host plant

However we can differentiate between latency and masking In the latter state the virus is presumably slightly different from the normal Although we have discussed the lysogenic bacteria under latency they should perhaps be more correctly dealt with under "masking Since no phage particles can be demonstrated in the lysogenic bacteria the virus is presumably in a different physical state Similarly with the Shope papilloma virus and that of swine influenza in these cases also the viruses seem to be in a noninfectious condition

We can say tentatively then that we have *latency* a condition caused solely by the host virus relationship further we have *masking* where the virus is actually in a different state There is the prophage which is presumably an early or resting stage of development and there is a noninfectious stage in some viruses which possibly is caused by a temporary combination of the virus with host-cell elements

We do not know at present how other incomplete virus forms fit into the picture such as occur with the turnip yellow mosaic and tobacco mosaic viruses and some insect viruses

BIBLIOGRAPHY

Andrewes C H *In* A System of Bacteriology vol 7 p 311 London His Majesty s Stat Off., 1930

—— *In* Advances Virus Res 4 1 1957

—— Proc Roy Soc Med 33 75 1939

Bawden F C. Nature 177 302 1956

Beard J W Cancer Res 16 279 1956

Bennett C W Phytopathology 34 77 1944

Burnet F M Virus as Organism p 5 Cambridge Mass Harvard 1945

Carr J C Brit J Exper Path 23 ? 1 1942

—— Symp Interaction of Viruses and Cells p 97 VIth Internat Congr Mi rol ol., Rome 1953

Cole R and Kuttner A G J Exper Med 44 855 19 6.

Duran Reynals F Ann New York Acad Sc 54 977 1952
Gledhill A W and Dick G W A J Path & Bact 69 311 1955
Gregoire C J Gen Microbiol 5 121 1951
Holmes F O Phytopathology 24 845 1934
Kassanis B J Gen Microbiol 15 620 1956
Kidd J G and Rous P J Exper Med 67 399 1938
Luria S E General Virology New York Wiley 1953
Lwoff A *In* The Nature of Virus Multiplication London Cambridge 1953
Lwoff A and Guttman A Ann Inst Pasteur 78 711 1950
Lwoff A Siminovitch L and Kjeldgaard N Ann Inst Pasteur 79 815 1950
Peacock A and Peacock P R *Brit J Cancer* 3 289 1949
Rous P Am J Cancer 28 233 1936
Rous P and Kidd J G J Exper Med 68 529 1938
Salaman R N Phil Tr R Soc B 229 137 1939
Salaman R N and LePelley R H Proc Roy Soc B 106 140 1930
Selbie F R *Brit J Exper Path 27 143 1946*
Selbie F R Robinson R H M and Shope R E Brit J Cancer 2 375 1948
Shope R E J Exper Med 65 219 1937
——— *In* Viruses p 79 California Inst Tech 1950
Smith K M Nature 167 1061 1951
——— Parasitology 37 126 1946
——— Parasitology 37 131 1946
Smith K M and Rivers C F Parasitology 46 235 1956
Traub E J Exper Med 69 801 1939
Watson M A Ann Appl Biol 44 599 1956
Yamafuji K Enzymologia 15 223 1952

Viral Inhibition

DR THOMAS FRANCIS JR

In opening the subject of viral inhibition I present broad concepts rather than attempt a detailed review of the manifold studies and the intricate biochemical reactions involved They have been covered extremely well in recent excellent reviews and symposia I will try to provide a prospectus as to how viral inhibitors might be introduced to the control of a human virus disease and to consider current indications of the feasibility The discussion which has been arranged to follow can be expected to amplify or emphasize features of special interest

Virus inhibition has varied connotations including (a) in vitro serologic reactions (b) the normal physiologic influences or barriers and (c) those induced alterations in reactivity which are generally designated chemotherapy or chemoprophylaxis Study of viral inhibition also is approached variously through investigations at the molecular or cellular level at the more complex level of the organ usually affected or in the intact animal where primary cellular infection virus distribution beyond the primary site and pathognomonic localization in a more distant vulnerable cell or organ may be progressive stages of infection leading to gross disease in a cellularly complex bu integrated host

The advantage of having extensive knowledge of the pathogenesis of a disease even though areas of uncertainty do exist as a basis for reasoned approaches to chemoprophylaxis and therapy is well illustrated by poliomyelitis Studies in man and other primates seem to have established that poliomyelitis is ordinarily an infection of the alimentary tract and that the entry of virus to nerve cells takes place by vascular extension of virus from the alimentary tract Therefore I shall proceed to the outlook for viral inhibition using poliomyelitis the subject of this Conference as an illustrative text

In this process one can visualize multiple sites at which viral infection proceeding from benign cellular involvement to severe disease theoretically may be inhibited by

(1) Preventing the union of virus presumably introduced by mouth with susceptible cells in the alimentary tract

(2) Preventing (a) the penetration and (b) the development of virus in these cells after union has occurred

(3) Eliminating established infection in the alimentary tract

(4) Limiting the multiplication of virus to the alimentary tract so that it does not extend

(5) Intercepting virus in its distribution by vascular systems (blood or lymph) so that it does not arrive in the central nervous system

(6) Limiting or eliminating extension of virus along nerve fibers

(7) Altering nerve cell so as to *prevent* essential injury even though its peripheral fibers are infected

(8) Inducing changes in nerve cells so as to *limit* injury and aid recovery

(9) Acting on virus itself after infection is established in the nerve cell

(10) Preventing the extension of virus from infected damaged cells to other susceptible neurones

These are general propositions not implying precise information of the mechanisms involved at the various stages

What suggestions are there that these stages can be inhibited? What effect could such inhibition have not only on individual cases but also on the epidemiologi aspe ts of the disease? How could experimentally effective materials be made available to the population?

1 One possibility for preventing the union of virus with susceptible cells of the alimentary tract would be the establishment prior to exposure to the virus of a *virucidal* activity in the secretions or tissue fluids at the portal of entry similar to the prophylactic use of sulfonamides or penicillin against bacterial infection

An alternative would be to make available at the portal of entry a substance which not virucidal of itself would prevent the combination of virus with suitable cells This appears to be the effect of certain a amino sulfonic acids which prevent the effective union of influenza virus with susceptible cells but does not destroy the virus the virus apparently perishes from inhospitality Recently indications are that the effect of the sulfonic acids results from interference with potassium availability recalling the requirement of certain bacteriophages for tryptophane or inorganic ions to achieve their attachment Could chelating agents produce a similar effect? The pectin polysaccharides apparently interfere with virus attachment to the cellular substrate Another possibility is inhibition of a toxic component of the virus which by producing injury at the cell surface may be a significant factor in the efficiency of the virus to combine *with cells The destruction of replaceable cell* receptors by RDE is another factor It is seen then that there are indications that inhibition of cell virus union can be accomplished

2 Attachment of virus occurs rapidly but does not immediately render virus insusceptible to extracellular influences Moreover much of the virus which combines with the cell may fail to gain entrance Thus substances which would delay or further reduce incorporation of virus with the cell could be sought They could result either in virus disintegration or in rendering it more readily disposable by the body Virus interference may have such an effect Here enhancement of normal physiologic inhibitors may be of importance since they tend to increase spontaneously with injury It seems likely that inhibition of viral progress at this introductory stage is achieved commonly by natural defense mechanisms in view of the high proportion of exposed subjects who escape without evidence of effective contact However Bawden has expressed the opinion that most inhibitors of plant virus infection act more on the cell to delay entry of virus or to stimulate cells to an unusual activity which renders virus noninfective He mentions that leaves treated with trichothecin after presumed infection are refractory but regain susceptibility in about 2 days which roughly resembles the effect of RDE on cellular receptors This stage of infection that is primary cellular penetration is under usual conditions an unrecognized period except during epidemics or situations of well identified exposure as with measles smallpox or rabies Therefore ordinarily an effect on this early phase almost of necessity would have to be pre-established and induced alterations in the cells would have to be physiologically endurable for considerable periods of time In that respect the first two steps almost may be looked at as the same problem The outlook is essentially the prevention of infection

Prevention of the development of virus which has become vitally established in the cells is the focus of much attention Tissue cultures are especially suitable to this type of investigation since they permit close observation of growth curves under relatively well-defined conditions Great numbers of materials have been tested antimetabolites of many kinds metabolic analogues dyes energy and oxidation inhibitors *antibiotics polysaccharides and synthetic poly* peptides Examples of nearly all of these classes have been shown to exert an effect in limiting infection and the yield of assorted viruses Metabolic analogues are of special interest since they permit identification of specific effects by their specific reversion by the normal compounds Their use has indicated the practicality of metabolically blocking the formation of virus under physiologically tolerated conditions They also permit designation of distinct steps in virus development and demonstrate the susceptibility of the different steps to different influences They have one disadvantage in that resistance to the metabolite commonly develops The studies with methoxinine and fluorophenylalanine have been among the most informative concerning animal viruses those with benzimidazole *derivatives* have been among the most consistently progressive in designing compounds of increasing effectiveness Although there has not yet emerged a clear design for integrated interpretation of the numerous observations they emphasize repeatedly that intracellular virus infection can be influenced to the advantage of the host by induced *alterations in the metabolic processes* of the cell One might interpret that this may be accomplished much more readily in a specific basic type of cell like the lymphocyte The results also point out that the cell is not merely a subservient biochemical community irrevocably at the whim of a dictatorial virus

that the cell is not entirely a passive participant in virus union penetration and development

The possibility has been expressed that cells disturbed by virus infection may be detected selectively by chemical compounds and destroyed so that developing virus also succumbs If this occurred to a limited extent in the superficial or lymphatic cells of the alimentary tract bearing the initial infection theoretically no major harm would be done However if anterior motor neurons showing evidence of injury were destroyed an exaggerated paralytic disability would ensue

This like the 2 preceding steps represents prevention of virus establishment preparation for which would be required in advance How could this be accomplished practically? One readily can visualize the administration of an effective chemical to the general population by distributing it throughout the poliomyelitis season in some simple vehicle like iodide in salt fluoride in water or reinforcements in bread or milk

3 Most biochemical studies have been directed to un-co-ordinated virus-cell systems resulting in disruption of the cell However virus infection is commonly of such a nature that the virus does become sufficiently co-ordinated with the synthetic activities of the cell to create an integrated cell virus relationship which can persist

To some extent this appears to be the situation encountered in continued infection of the alimentary tract where Podian s data indicate that the lymphoid tissue is heavily infected without obvious anatomic alterations The ability to eliminate that infection at will could be a valuable asset epidemiologically in reducing opportunity for transmission and clinically by reducing the probability of extension of the virus to the CNS so as to produce paralytic disease The studies of *Ward et al have suggested that certain mercurials can be effective in reducing alimentary virus infection However note that* Koprowski and his associates were unable by administration of calomel to eradicate the alimentary virus infection being maintained in human subjects after feeding Type I polio virus Nevertheless observations with other compounds such as betapropriolactone or certain detergents warrant further exploration into the destruction of alimentary-excreted virus. This may be of great value *not only re polio virus* but also re ECHO and Coxsackie agents But inevitably treatment of a well balanced infection of this nature will require particular consideration of location of the infection including the character and the function of the cell and the tissue involved However if poliomyelitis is primarily a virus infection of the lymphatic cells the specificity should increase the probability of discovering selective inhibitors of continuing virus development However prompt elimination may necessitate a multiple approach which will affect cell permeability produce a rapid metabolic imbalance and inactivation of the exuding virus Cortisone might be suitable as a disturber of the truce Specific antibody may well be used as an adjunct in this objective although the persistence of alimentary infection strongly suggests that it is in an extravascular location

4-5 The next approach is that if alimentary infection is not eradicated it should be limited sharply There are numerous recommendations for the idea of intercepting virus being released by cells of the alimentary tract which may by vascular dissemination reach the important motor neurons Much of this virus is extracellular and not yet in position to combine immediately with the specific vulnerable cells Therefore it should be more subject to combination with inhibitors or inactivators There are strong indications that it is true The influence of antibiotics such as helenine and 8450 on MM and Semliki Forest viruses in mice suggests that it is a viremic stage or a stage leading to viremia which is interrupted after peripheral introduction of virus. Both these materials given parenterally have a protective effect on monkeys infected with poliomyelitis virus either subcutaneously or by feeding There is some evidence that the production of virus in the alimentary *tract is reduced* but the action is not a direct action on virus in the alimentary tract as shown by the *fact that giving the antibiotics by mouth* has no effect on development of paralysis Therefore it must limit the dispersion of virus in the body Monofluoroacetate is a compound which was shown to reduce the extent of viremia in monkeys while providing protection against paralytic disease The effects observed with Atabrine are most distinct with the viremi neurotropic infections of mice induced intra*muscularly Probably some of the reported*

effects with dyes may be exerted on virus in the blood stream

With many other compounds the results noted either positive or negative with different viruses and routes of virus administration contain too many variables to permit a significant impression as to site of effect. Other materials under study in our laboratory give promise of significant protective effect against poliomyelitis in monkeys again with influence directed against circulating virus

Matthews has demonstrated that the guanine analogue 8 azaguanine when sprayed on leaves as late as 2 days after infection will prevent systemic development of certain plant viruses but it or other compounds were without effect after systemic involvement had begun He also noted that the analogue was incorporated into the virus formed early in the infection with a marked reduction in its infectious capacity This suggests that the influence of metabolic inhibitors is not necessarily limited to the cell only As suggested earlier it seems likely that vaccination with inactive virus exerts its major influence against paralytic poliomyelitis by neutralizing virus which may be circulated by the antibody developed It does not prevent the alimentary infection but protects the nervous system The effect of vaccine strongly supports the outlook for chemical inhibition of poliomyelitis virus in the vascular systems

6–10 The next possible sites of viral inhibition considered are in many respects reconsideration of the steps involved at the portal of entry except that they now refer to a second stage in pathogenesis in which specific nerve cells are the target Stepwise these are advanced stages of virus infection but may be considered to be preliminary stages of paralytic poliomyelitis Where and how do the nerve fibers and the vulnerable nerve cells become infected? Bodian suggests that it may naturally occur by overflow of virus into the area postrema Certainly there are strong indications that under certain conditions—tonsillectomy operation overactivity inoculation—the virus may proceed directly along fibers toward the neurone The effect of sectioning the nerve injections of alcohol cocaine or other materials which alter its physiologic state may reduce extension along the nerve Thus substances which have a direct inhibitory or mimetic influence on nerve physiology are of interest or substances acting through the main cell body may be influential.

The stages discussed earlier can be presumed to be prophylactic in terms of paralytic disease although therapeutic of the infection Any procedure which can reduce quantitatively the number of neurones affected or the severity of injury to the neurones may be of importance in therapy of paralytic poliomyelitis One is faced with ignorance of the mode of entrance of the virus into the nerve cell or of the biochemical mechanisms of the injury The destroyed cells cannot be expected to regenerate But from Bodian s observations 80 per cent of the neurones which histologically appear to be affected may recover and this points to the possibility that chemotherapy may be able to speed that restoration In fact the question is whether much of that injury is a toxic effect as has been demonstrated by Ackermann Payne and Kurtz in tissue culture rather than a result of free virus multiplication In this event substances like Xerosin which reduce toxic effect of viruses could be useful Or are these restorable neurones ones whose infection has been interrupted in the peripheral processes?

If knowledge of the substrate with which poliomyelitis virus combines in the cell can be obtained it may be possible to prepare a protective inhibitory analogue This possibility is implicit in our demonstration of a protein in the gray matter presumably in the neurones of the CNS which combines selectively with poliomyelitis virus The studies of Hyden of the effect of malononitrile on the nucleoprotein of the motor neurones led to examination of that compound s influence on experimental disease Although at times some influence was noted malononitrile gave no significant protection Finally if a limited number of cells is initially infected the introduction of inhibitors which prevent further development of virus or attachment to new cells could at this stage be beneficial

This broad view of approaches to viral inhibition in terms of the mechanisms of pathogenesis of a virus disease has served primarily to emphasize that the outlook does not have to be limited to one theme or one stage of infection The point of view also may shift according to whether the intent is prevention or treatment Clinically the term preventive or prophylactic can be

applied to any effect attained prior to onset of manifest disease and therapy only when disease has become apparent Experimentally when the time of infection is known it is our tendency to call prophylaxis any effect obtained in animals by administration of the test material before infection and therapy effects gained after infection In tissue-culture systems this same experimental designation applies

At this point I make a few remarks about the test systems The use of tissue or cell cultures has obvious advantages They have permitted much learning of the different stages in viral development and how they can be inhibited But this information has not ordinarily sustained its promise when tested in the complex medium of the susceptible animal That failure points to the difficulty of attaining in one the conditions readily encountered in the other In cultures the cells or tissues are separated from the controlling influences of the total organism they are likely to be of a type quite different from the highly differentiated cells of the body characteristically damaged by the virus under study The system has probably become adjusted so that virus readily encounters suitable cells and an abnormally brief cycle of virus development occurs so that virus might be more difficult of inhibition The virus may be changed so that it is no longer like the natural prototype Consequently greater attention to these factors might lead to better translation of effects to the whole animal

CONCLUSION

The best composite view of viral inhibition is that presented by the naturally resistant species. This state seems strongly to recommend greater effort to understand the conditions which govern that inhibitory action of the nature of varied serologic influences natural antibody information of the behavior of cells in animals with solid or partial natural immunity vis à vis the susceptible These need study for understanding of cell virus relationships I emphasize once more a thesis that I have presented repeatedly— that in study of viral inhibitors we must pay more attention to identification of the virus action and its specific substrate we must recognize that the cell is not a passive fixture in this reaction and that fixation and penetration of virus may be more the active operation of the cell than of the virus Finally in contrast with the outlook commonly followed for cancer chemotherapy our purpose is not to destroy these highly necessary cells but to support and restore them

BIBLIOGRAPHY

Ackermann W W Proc Soc Exper Biol & Med 80 363 1952

Ackermann W W and Maassab H F J Exper Med 102 393 1955

Ackermann W W Payne F E and Kurtz H J Exper Med (In Press) 1958

Bawden F C Advances Virus Res 2 31 1954

Bodian D Am J Hyg 55 414 1952

—— Am J Hyg 64 181 1956

—— Poliomyelitis Papers and Discussions Presented at the Second International Conference p 61 Philadelphia Lippincott 1952

Cochran K W Brown G C and Francis T Jr Proc Soc Exper Biol & Med 85 104 1954

Cochran K W and Francis T Jr Proc Soc Exper Biol & Med 92 230 1956

Fazekas de St Groth S Australian J Exper Biol & M Sc 26 271 1948

Francis T Jr Brown G C and Kandel A Proc Soc Exper Biol & Med 85 83 1954

Francis T Jr and Chu L W Atti 6 Congr Internat Microbiol 3 167 1953

Green R H and Wooley D W J Exper Med 86 55 1947

Groupe V and Herrmann E C J Immunol 74 249 1955

Hurst E W Peters J M and Melvin P Brit J Pharmacol 7 473 1952

Hyden H., and Hartelius H Acta psychiat et neurol (Kbh Supp) 48 1 1948

Koprowski H South African M J 29 1134 1955

Levine A S Bond P H Scala A R and Eaton M D J Immunol 76 390 1956

LoGrippo G A Earl D P., Jr Graef I., and Ward R Am J Hyg 55 70 1952

Matthews R E F J Gen Microbiol 8 277 1953

—— J Gen Microbiol 10 521 1954

Powell H M Culbertson C G McGuire J M Hoehn M M., and Baker L A Antibiotics & Chemother 2 437 1952

Shope R E J Exper Med 97 601 and 627 1953

Tamm I Folkers K., and Shunk C H J Bact 72 54 1956

Ward P., LoGrippo G A., Earl D P., Jr Graef I Fed Proc 12 464 1953

DISCUSSION

Dr Dulbecco Poliomyelitis viruses allow the study of the genetic properties of a biologic system having ribonucleic acid as the only or main nucleic acid This study is particularly interesting since all the systems the genetics of which is fairly well understood contain deoxyribonucleic acid A model for the latter systems are the bacteriophages the genetics of which has been discussed today by Delbruck

Our laboratory has been engaged in the study of poliovirus genetics for some time and a number of results have been secured The work that I am going to discuss briefly concerns essentially a study of the mutability of polio virus both spontaneous and induced

SPONTANEOUS MUTABILITY

The spontaneously occurring mutants which were isolated and studied in our laboratory are

1 Heat resistant mutants They differ from wild type virus for their smaller rate of inactivation under identical conditions Different types of these mutants occur having different inactivation rates A recent finding may lead to an understanding of the structural changes produced by the mutations In fact in collaboration with P Pohjampelto we have recently discovered that the sensitive virus can be made as resistant as the most resistant mutant by exposure to cystine

2 Mutants having a reduced efficiency of plating under an acid overlay They are called *d mutants* where d stands for delayed plaque appearance The wild type virus which has a high efficiency of plating under acid agar is called d^+ These mutants have a special interest because they are frequently attenuated for the nervous system of the monkey although with some exceptions

3 Mutants having different plaque sizes similar to those described by Sabin The interaction between these mutants was studied They occur independently since most of the possible combinations were obtained by successive mutations However there are some distinct interactions Thus it was impossible to isolate a temperature resistant d mutant whereas temperature resistant d^+ mutants can be isolated very easily Perhaps the former combination is lethal or the expression of one of the characters is suppressed by the other Another example of interaction is the decreased d character of large plaque mutants of d virus

The d mutants give rise to reversions in the d^+ direction A quantitative study of the d to d^+ mutations can be carried out since by plating on acid agar the reverted virus particles can be detected and counted when they occur in a proportion of 1 in 1 000 000 of d particles By using this system it was possible to prove by standard microbiologic technics (the fluctuation test of Luria and Delbruck) that the d^+ reversions occur spontaneously at any time during the growth of the d virus The mutation frequency of certain mutational steps could also be measured by the same technic and was found to be of the order of 1 in 100 000 d particles per viral duplication

The expression of the d character can be measured in a quantitative way Such a measure is given by the ratio of the virus titer on acid and alkaline plates respectively and is defined as the efficiency of plating acid The efficiency of plating acid can vary between 1/1 000 or 1/10 000 for high-efficiency d mutants and 1/1 000 000 for low-efficiency mutants The reversions obtained from high-efficiency and low-efficiency mutants (respectively) have different properties as will be shown presently High efficiency mutants revert apparently in 1 step to virus which is very similar to wild type in d character In collaboration with H A Wenner we could prove that the reverted virus is also similar to wild type in neuropathogenicity Low efficiency d mutants revert to types intermediate in d character By continued selection several intermediate types of increasing d^+ phenotype can be isolated however a real d^+ virus is never obtained In collaboration with A B Sabin we could show that these reversions maintain the character of attenuation

Due to the extensive correlation between d character and neuropathogenicity it is likely that in these cases the d mutants are due to

genetic changes affecting loci which control both characters simultaneously The following picture may be suggested

The high-efficiency d mutants are more complex since they do not give rise to complete reversions with a measurable frequency This fact and the more pronounced phenotypic effect that they display both in relation to d character and neuropathogenicity suggest that they may be deletions at a d locus The reversions for the d character of these mutants can be attributed to mutations at suppressor loci which affect only some of the phenotypic effects of the deletion

Now consider the problem of the induction of mutations in polio virus by mutagenic agents Experiments on mutagenesis could be carried out by using the mutations from d to d^+ of a high-efficiency d mutant Three agents so far were investigated x rays ultraviolet light and proflavine Either radiation was used to irradiate virus-infected cells rather than free virus since previous work with bacteriophages had shown that this was the most suitable way for inducing viral mutations Although a large variety of doses of radiation was used and the irradiation was given at various times during the latent period no mutagenic effect was observed in either case Proflavine on the contrary was found to be effective Infected cells were exposed to different concentrations of the drug during the whole period of virus multiplication (about 24 hours) At adequate concentrations of proflavine the total virus yield from these cells was depressed and the proportion of mutants in the yield was increased the 2 effects being correlated The maximum effect obtained was a nearly tenfold increase in the proportion of mutants over background A selective effect of proflavine on the mutants was excluded We do not know as yet whether proflavine is effective in increasing the proportion of other mutations as well However since mutagenic effects are generally non specific it may be suggested that a proflavine treatment should precede any attempt to isolate mutants of poliomyelitis virus endowed with special genetic properties

We add the following concluding remarks One of the facts that begins to emerge from the reported studies and others which were not discussed here is that the genetic and reproductive properties of poliomyelitis viruses do not differ fundamentally from those of some DNA viruses such as the even-numbered bacteriophages of *E coli* It is particularly striking that the same agents are mutagenic in both cases The observed similarities suggest that although the 2 types of viruses have a different nucleic acid they may have some very similar step in their multiplication

BIBLIOGRAPHY

Luria S E and Delbruck M Genetics 28 491 1943

Sabin A B J A M A 162 1589 1956

Vogt M Dulbecco R and Wenner H A Virology 4 141 1957

Dr Schafer In his impressive review Dr Horsfall pointed out that ribonucleic acid (RNA) is sufficient to cause the formation of new virus particles not only in the tobacco mosaic virus but also in the Mengo encephalitis virus

In addition an infectious principle having properties similar to the material obtained with the Mengo virus was isolated recently at our Institute from the United States strain of equine encephalomyelitis (Eastern type)[1] According to experiments carried out thus far it can induce the formation of new infectious particles both in the brain of mice and in the allantois of chick embryos However it is quite distinct from the usual infectious agents on account of its sensitivity with respect to ribonuclease its lability in the presence of high temperatures and its behavior when precipitated with alcohol (see Table 176) These are either partly de

TABLE 176 BEHAVIOR OF THE ENCEPHALOMYELITIS VIRUS AND OF THE ISOLATED RNA FRACTION

Treatment	Virus Preparation	RNA Fraction
	MID_{50} per cu cm (−log)	MLD_{50} per cu cm (−log)
Initial value	8 1	3 0
Ribonuclease	7 7	0
4 hours at 37 C	6 7	0
Alcohol precipitated	0	3 5

MID_{50} for chick embryos
10 eggs dilution

E ery 3 ... n tre ted w th ibonucl se

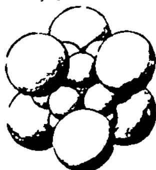
composed virus particles which the RNA has released or the RNA of the virus particle itself Further experiments to determine the characteristics of this material are now in progress

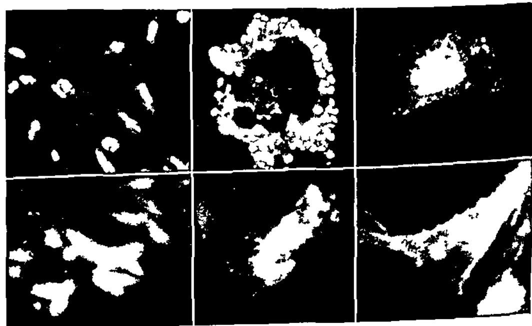
Our efforts to isolate a pertinent component in the virus of classic fowl plague—which has been investigated more intensively by us in the last few years and nucleic acid which as in the case of the virus of equine encephalomyelitis is present only in the form of ribonucleic acid —have thus far persistently failed This may be due to the fact that the experimenter could not reproduce the invading mechanism of the structurally more complex virus of fowl plague

However it has been possible to obtain by other means a considerably more complete idea of the course and the nature of the reproduction of this virus

In order to present these results I shall discuss briefly the structure of the virus particle of classic fowl plague As shown in the diagram at the top of Figure 227 it consists of at least

FIG 227 (*Top*) Diagram of the virus of classic fowl plague (*Center left*) Embryonal chick cells 3 hours after infection Fluorescence of the nuclei after treatment with fluorescent B antigen antibody (*Center middle*) Chicken macrophage giant cell 3½ hours after infection Fluorescence of the nuclei after treatment with fluorescent B antigen antibody (*Center right*) Embryonal chick cell 10 hours after infection Fluorescence of the nucleus and of the cytoplasm after treatment with fluorescent B antigen antibody (*Bottom left*) Embryonal chick cells 4 hours after infection Fluorescence of the entire cell with greater fluorescence at juxtanuclear points Fluorescent hemagglutinin antibody (*Bottom middle*) Chicken macrophage giant cell 4 hours after infection Fluorescence at the cytoplasm Nuclei blank Fluorescent hemagglutinin antibody (*Bottom right*) Embryonal chick cell 14 hours after infection Greater fluorescence at the cell periphery fluorescent protrusions Fluorescent hemagglutinin antibody





2 types of subunits of which the internal bound or B antigen is the bearer of the RNA of the virus particle whereas the hemagglutinin which lies on its surface does not contain any nucleic acid but does possess the receptor-destroying enzyme and the adhering groups of the virus [7 9] In regard to all the properties tested the S-antigen encountered outside the virus particle corresponds to the B antigen [8]

It could be shown with the aid of virus particles whose B antigen was labeled by incorporation of P^{32} into its RNA that this virus too—just as the influenza virus[6]—disintegrates when the host cell is infected releasing its B antigen and from this probably even the *RNA* [11]

The resulting formation of new virus material in the cell was recently investigated by means of fluorescent antibodies[3] in infected tissue cultures [1 4] The impression was gained that the B antigen is formed in the nucleus It can be demonstrated there first 2 to 3 hours after the infection (Fig 227 *center left and middle*) Later it is also observed in the cytoplasm (Fig 227 *center right*) The hemagglutinin which appears an hour later than the B antigen is present right away throughout the cytoplasm (Fig 227 *bottom left and middle*) and is encountered in higher concentrations in a juxtanuclear position the significance of which is not yet clear to us at this time It later accumulates along the periphery of the cell where it also can be demonstrated in the form of fine protrusions (Fig 227 *bottom right*) We know from ultrahistologic investigations that it is only here that the new virus particles are formed

The observations described indicate that the two known components of the classic fowl plague virus are formed not only at different times but also at different places in the host cell

REFERENCES

1 Breitenfeld P M and Schafer W The formation of fowl plague virus antigen in infected cells as studied with fluorescent *antibodies* Virology 4 328 1957

2 Colter J S Bird H H, and Brown R A Infectivity of ribonucleic acid from Ehrlich ascites tumour cells infected with mengo encephalitis Nature 179 859 1957

3 Coons A H and Kaplan M H Localization of antigen in tissue cells J Exper Med 91 1 1950

4 Franklin R M The growth of fowl plague virus in tissue cultures of chicken macrophages and giant cells Virology (In Press) 1958

5 *Hotz G., and Schafer W Ultrahistological* study on virus reproduction in classic fowl plague Ztschr Naturforsch 10b 1 1955

6 Hoyle L. and Frisch Niggemeyer W The disintegration of influenza virus particles on entry into the host cell studies with virus labelled with radio-phosphorus J Hyg 53 474 1955

7 Schafer W Units isolated after splitting fowl plague virus *in* The Nature of Viruses p 91 Ciba Foundation Symposium London Churchill 1957

8 Schafer W Munk K and Mussgay M Physico-chemical and biological properties of the purified soluble antigen of classic fowl plague Ztschr Naturforsch 11b 330 1956

9 Schafer W and Zillig W On the structure of the elementary virus particle of classic fowl plague I Production physico-chemical and biological properties of some cleavage products Ztschr Naturforsch 9b 7 9 1954

10 Wecker E and Schafer W An infectious component of ribonucleic acid principle from the virus of the United States strain of equine encephalomyelitis (Eastern type) Ztschr Naturforsch 12b 415 1957

11 ——— Studies with P^{32} labelled virus of classic fowl plague Ist communication Ztschr Naturforsch 12b 483 1957

DR TAMM The manifold aspects of the process of virus multiplication and the various approaches to virus inhibition have been set forth comprehensively by earlier speakers I confine my remarks to approaches to selective inhibition of virus multiplication through chemical interference with the biosynthesis of nucleic acids or proteins

It is generally agreed that the process of virus multiplication is closely linked to the metabolic activities of host cells The fact that synthesis of virus materials inside susceptible host cells takes place in the absence of a limiting virus membrane reflects the intimacy of the host virus relationship This situation is underlined by the fact that all of the chemical compo

I Germ n C t k n p Hy Fr k b I nt n

have been used to inhibit virus multiplication have been found to have some effects on host cell metabolism at virus-inhibitory concentrations wherever such effects have been looked for In spite of this certain compounds are sufficiently active and selective to reduce the yield of virus in intact animals and to protect them against death from virus infection under restricted experimental conditions

Both the compound and the virus employed are important determinants in experiments on inhibition of virus multiplication Depending on the *chemical structure and biochemical* mechanism of action different chemical compounds interfere preferentially with different metabolic reactions Furthermore definite differences exist among virus-inhibitory compounds with respect to their activity and toxicity

In studies on virus inhibition with benzimidazole derivatives we have found that virus-inhibitory activity and toxicity of compounds may vary independently certain modifications in the structure of the inhibitor molecule have resulted in a disproportionate increase in influenza virus inhibitory activity relative to toxicity Among such derivatives showing enhanced selectivity are the β linked D ribofuranosides of halogenated benzimidazoles These compounds are among the few which are sufficiently active and selective to cause appreciable inhibition of influenza virus multiplication in mice and chicken embryos These ribosides of halogenated benzimidazoles are new inhibitors of ribonucleic acid biosynthesis and they appear to *act as antagonists of a natural purine riboside* Any departure from the ribose structure in the pentose moiety results in reduced influenza virus inhibitory activity and selectivity

The nucleic acid in influenza virus particles is of the ribonucleic acid type With the aid of the riboside of dichlorobenzimidazole it has been shown that virus nucleic acid synthesis precedes the formation of the more complex viral structures *such as the soluble complement* fixing antigen and virus particles

The nucleic acid in poliomyelitis virus is also of the ribonucleic acid type and as was expected the ribosides of halogenated benzimidazoles proved to be active as inhibitors of poliomyelitis virus in monkey kidney cells in vitro Studies with poliomyelitis virus were carried out in collaboration with Dr Marjorie M Nemes It should be emphasized that the ribosides were less active against poliomyelitis virus than against influenza virus Since both poliomyelitis and influenza viruses are ribonucleic acid viruses and since the ribosides of halogenated benzimidazoles reduce the rate of synthesis of ribonucleic acid an explanation for this difference in activity must be sought in the circumstances which surround the synthesis of virus materials inside host cells

At the higher concentrations required to inhibit poliomyelitis virus the ribosides were toxic *to cells Unexpectedly another glycoside of di*chlorobenzimidazole namely the α linked D-arabinopyranose derivative did show enhanced selectivity against poliomyelitis virus To elucidate the biochemical basis for the difference in selective action we have studied the effects of the riboside and the arabinoside on ribonucleic acid and protein synthesis in monkey kidney cells Although both compounds inhibited ribonucleic acid synthesis at concentrations inhibitory for the poliomyelitis virus the arabinoside which is more selective for this virus also had a marked inhibitory effect on protein synthesis *Furthermore compared with the riboside* the arabinoside could be given later after inoculation of virus and inhibition still obtained in fact some effect was obtained even after logarithmic increase in virus had begun

These observations suggested that inhibition of protein synthesis provided an approach to the selective inhibition of poliomyelitis virus multiplication which was preferable to inhibi*tion of ribonucleic acid synthesis This tentative* conclusion has received additional support from results of more recent experiments with 2 entirely different compounds azaserine an inhibitor of ribonucleotide synthesis and p-fluorophenylalanine an inhibitor of protein synthesis

The available inhibitors of protein synthesis in mammalian cells are all compounds of rather low activity on a weight basis For this reason alone they do not offer *promise of effectiveness* in intact animals It is to be hoped that through discovery or development new and more active inhibitors of protein synthesis will become available for studies on virus multiplication and other important biologic processes

Dr Zinder Dr Delbruck has told of some of the wonderful and extraordinary things that

occurred during the vegetative growth of bacterial viruses There are two things that a bacterial virus bacteriophage may do to a bacterium other than destroy it For this we need the class of bacteriophage called "temperate which do not destroy all of the infected organisms but rather in some fixed proportion dependent on the host the nature of the bacteriophage and the environment induce a lysogenic or carrier state

I digress for a few moments with regard to the phenomenon of lysogeny Following infection with a temperate bacteriophage almost all of the organisms survive and continue to grow but release during growth with a fixed frequency per generation a bacteriophage Therefore these bacteria carry the potentiality to produce bacteriophage at some later time This potentiality has been called prophage In general it is now known that a cell can carry only a single prophage of a particular kind and in all probability this prophage is carried on or in the bacterial chromosome The most important evidence of this comes from studies on *E coli* where in crosses between lysogenic and nonlysogenic bacteria prophage segregates as to the other bacterial markers and in fact turns out to be linked to other bacterial markers The lysogenic bacterium exhibits the property of being immune to superinfection by the identical phage which it is carrying This then is one property of a phage infected bacterium not of the kind of which I shall speak today We need such a system in order that the bacteria should survive the infection

I restrict this discussion to one such system—the one that I have been working with—and our host will be the mouse pathogen *Salmonella typhimurium* and a temperate bacteriophage which we have called P22 For the sake of simplicity and clarity we will reverse the chronology in the study of these phenomena

The first phenomenon has been called lysogenic conversion that is lysogenized cells exhibit a new property which is not obviously related to the presence of the prophage The most striking example which was discovered in modern times is that of the diphtheria organism which produces the diphtheria toxin only when it is lysogenic and does not produce it when it is nonlysogenic although again deferring to our human's historical sense probably there were reports of similar phenomena in the 1920s when it was reported that the streptococci producing scarlet fever were also lysogenic and the erythrogenic toxin probably was controlled by the presence of bacteriophage

In our system—the *Salmonella typhimurium* system—when the cell becomes lysogenic for the bacteriophage P22 it is not only immune to secondary infection but also exhibits a new somatic antigen This somatic antigen is the somatic antigen 1 which long has been known in the bacteriology of *Salmonellae* and has been used as a diagnostic criterion of the antigenically complex *Salmonellae* About 10 minutes following infection with the bacteriophage the antigen is sufficiently developed to be detected by the rather crude serologic means of cell agglutination When lysogenic bacteria are cured of the prophage as they can be by appropriate ultraviolet treatment the cells eventually will lose this antigen Thus every lysogenic cell exhibits this property and loses it following loss of the phage

The other phenomenon mediated by bacteriophage is what we have called transduction Herein following infection by a temperate bacteriophage a small proportion of the treated bacteria is altered in such a way as to be a reflection of the genetic constitution of the host upon which the phage last grew Let me cite an example

Given a donor bacterium which is capable of fermenting the sugars galactose and xylose the phage is grown on such a strain Given a recipient which is incapable of fermenting either sugar either as obtained in nature or more readily obtained in the laboratory by sequential mutational events we then infect this recipient and among the progeny of these infected cells we find organisms which are capable of fermenting either sugar but not both They occurred with the approximate frequency of 1 in 1 000 000 per phage particle In the *Salmonellae* with this particular bacteriophage almost all bacterial properties can be transduced These include such properties as drug resistance nutritional requirements and flagellar antigens which do not seem to be under the direct control of lysogenic conversion This phenomenon of transduction is distinguished from general mating by the fact that only a part of the bacterial genome is transferred in any particular event

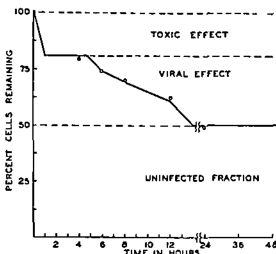


FIG 228 Data are a composite of two experiments Each point represents a single experimental culture Per cent cells remaining = 100 x cell count of experimental culture/cell count of control culture Each experimental culture was exposed to 7 4 PFU of virus cell for 15 minutes

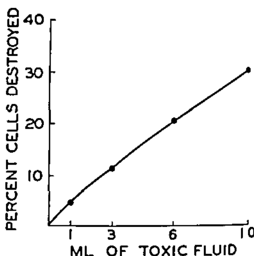


FIG 229 Replicate cultures of cells were exposed to 4 ml of balanced salt solution containing amounts of toxic fluid ranging from 0 1 to 1 0 ml After exposure for 30 minutes cultures were washed 5 times to remove the infecting fluid and incubated at 37 in maintenance solution for 45 minutes At the end of this period the surviving cells were determined The per cent of cells destroyed was determined by comparison with a control culture

Therefore we see that in addition to cytopathogenic effects of viruses at least some bacterial viruses are capable of producing 2 types of permanent modification and therefore probably genetic modification of their hosts In one the property seems to be controlled directly by the presence of the phage genome—this being the lysogenic conversion for loss of the prophage involves the loss of the property On the other hand in transduction it is probable that the virus can be viewed only as a passive vector of fragments of genetic material a kind of casual contamination of the virus I do not know of the existence of such phenomena in animal or plant viruses but so many of the technics that are used in bacteria are dependent on the ability to maintain pure clones of organisms and high use of selective procedures that the developments described here yesterday portend the discovery of similar and further phenomena in animal and plant viruses

DR ACKERMANN It is quite likely that when the useful application of viral chemotherapy is realized the essence of the principle involved will be found to have been considered in one of the 4 papers presented this morning At present it remains for some shrewdly discerning individual among us to recognize by foresight which principle is the essential one

For myself I was impressed with the diverse cell responses which Dr Smith described for the varied host virus interactions It is this type of observation of comparative virology which led us to question whether or not in a single host virus system viral multiplication and cell destruction result from autonomous reactions Indeed it has been possible to show that substances such as p-fluorophenylalanine which in tissue culture completely prevents increase of polio virus do not prevent infection nor alter the subsequent typical viral cytopathology A recent observation in our laboratory is pertinent in relation to this observed degree of autonomy

of the cytopathogenic effect produced in HeLa cells by infection with polio virus

A toxic effect of fluids obtained from tissue cultures infected with Type I polio virus was observed when the fluids were introduced into fresh cultures The destructive activity is distinct from the usual virus induced cytopathogenic effect in that it occurs rapidly without a prolonged latent period and without virus production This is illustrated in Figure 278 where the cytopathogenic activity of an infected tissue culture fluid on HeLa cells was followed quantitatively as a function of time The data show that a loss of cells from the glass substrate of the culture occurs within 2 intervals 1 within the first hour and the second between the sixth and twelfth hours Between the first and fourth hours and in the period from 24 to 48 hours the cell count was constant Under these conditions intracellular virus does not appear until sometime between the third and fourth hour after infection There is a distinct intracellular phase and release of virus from the cells begins between the sixth and seventh hour

The response of cultures to various concentrations of tissue-culture fluid was determined In Figure 279 the percentage of cells destroyed is plotted as a function of the concentration of the infected fluid present in the exposure period It is seen that at low concentrations of the range studied the response of cells was nearly linear while at the higher concentrations the response appeared to be diminishing The data strongly indicate that the rapid cell destruction is the result of a specific material in the fluid Dilutions greater than tenfold do not produce effects which are measurable with accuracy

The infectious activity can be neutralized selectively by certain antiserum with full retention of the toxicity i e with antiserum prepared by Wenner in monkeys using infected monkey cord material However antisera prepared with infected tissue-culture fluid also will neutralize the toxic effect The antitoxic effect of such sera may be selectively adsorbed with lyophilized preparations of HeLa cells but antibodies prepared against uninfected HeLa cells do not show an antitoxic effect further the two activities possess differential heat stability

It should be emphasized that the ability to destroy cells and give rise to detectable amounts of toxin is a characteristic of only certain lines of the Mahoney strain of Type I polio virus In a comparative study 2 lines were investigated these are designated PT and pt These lines are also distinct in their plaque morphology The PT line produces large rapidly forming plaques and gives rise to toxic tissue-culture fluids The pt line is characterized by small late forming plaques and nontoxic fluids

It is interesting to speculate that the toxic activity of the tissue-culture fluid observed in fresh cultures is the same which produced the cytopathogenic effect and accompanied virus production in the culture of its origin

Diagnosis

Friday Morning, July 12, 1957

Chairman

DR WILSON SMITH
Department of Bacteriology
University College Hospital Medical School
London

Speakers

DR EDWIN H LENNETTE
Viral and Rickettsial Disease Laboratory
California State Department of
Public Health
Berkeley

DR ALBERT B SABIN
Children s Hospital Research Foundation
Cincinnati

DR JONAS E SALK
Virus Research Laboratory
University of Pittsburgh
Pittsburgh

DR G L LE BOUVIER
Yale University School of Medicine
New Haven

DR JOSEPH L MELNICK
College of Medicine
Baylor University
Houston

DR GILLIAN CHURCHER
Department of Bacteriology
University College Hospital
Medical School
London

Discussants

PROF DR HANS BERNKOPF
Hebrew University
Hadassah Medical School
Jerusalem

PROF D BLASKOVIC
Czechoslovak Academy of Sciences
Institute for Virology
Bratislava

DR SVEN GARD
Karolinska Institute
Stockholm

DR JAMES H S GEAR
South African Institute for
Medical Research
Johannesburg

PROF E GRASSET
University of Geneva
Institute of Hygiene
Geneva

DR TORSTEN JOHNSSON
Karolinska Institute
Department of Virus Research
Stockholm

DR M S LIKAR
Mikrobioloski Institut
Ljubljana Yugoslavia

DR P N MEENAN
St Vincent s Hospital
Department of Pathology
Dublin

PROF F PRZESMYCKI
Institute of Hygiene
Warsaw

Diagnosis

Friday Morning, July 12 1957

Chairman

DR WILSON SMITH
Department of Bacteriology
University College Hospital Medical School
London

Speakers

DR EDWIN H LENNETTE
Viral and Rickettsial Disease Laboratory
California State Department of Public Health
Berkeley

DR ALBERT B SABIN
Children's Hospital Research Foundation
Cincinnati

DR JONAS E SALK
Virus Research Laboratory
University of Pittsburgh
Pittsburgh

DR G L LE BOUVIER
Yale University School of Medicine
New Haven

DR JOSEPH L MELNICK
College of Medicine
Baylor University
Houston

DR GILLIAN CHURCHER
Department of Bacteriology
University College Hospital Medical School
London

Discussants

PROF DR HANS BERNKOPF
Hebrew University
Hadassah Medical School
Jerusalem

PROF D BLASKOVIC
Czechoslovak Academy of Sciences
Institute for Virology
Bratislava

DR SVEN GARD
Karolinska Institute
Stockholm

DR JAMES H S GEAR
South African Institute for Medical Research
Johannesburg

PROF E GRASSET
University of Geneva
Institute of Hygiene
Geneva

DR TORSTEN JOHNSSON
Karolinska Institute
Department of Virus Research
Stockholm

DR M S LIKAR
Mikrobioloski Institut
Ljubljana Yugoslavia

DR P N MEENAN
St Vincent's Hospital
Department of Pathology
Dublin

PROF F PRZESMYCKI
Institute of Hygiene
Warsaw

DR JOSEPH E SMADEL
National Institutes of Health
Bethesda

DR ARNE SVEDMYR
Virus Department of the
Central Bacteriological Laboratory
Stockholm

PROF DR J D VERLINDE
Netherlands Institute for Preventive
Medicine
Leiden

DR M K VOROSHILOVA
Institute for Poliomyelitis Research
Moscow

Problems of the Viral Diagnostic Laboratory with Respect to Poliomyelitis

DR EDWIN H LENNETTE

Application of the newer tissue-culture technics to the laboratory diagnosis of poliomyelitis has not only greatly simplified the diagnostic methodology but also has made it possible to apply diagnostic tests on a scale impossible only a few years ago However procedural simplicity is not necessarily commensurate with diagnostic infallibility and each of the several tests currently in use has its own shortcomings Throughout this discussion I shall speak primarily from the viewpoint of a viral diagnostic laboratory and not from that of a viral research laboratory I mention this since it seems likely that in some respects the experience of research laboratories may not coincide entirely with ours I should like then to say something of the problems and the difficulties encountered by a laboratory which undertakes to assist the clinician in establishing or ruling out a diagnosis of poliomyelitis by virus isolation attempts and/or serum antibody assays

DIAGNOSIS BY ISOLATION OF VIRUS

Virus isolation is regarded as a more useful diagnostic test than either of the serologic methods since it affords a means of making an early diagnosis It is generally considered that through the use of tissue-culture technics the virus can be recovered from approximately 90 per cent of early paralytic cases or serologically confirmed nonparalytic cases if the stool specimens are collected properly and during the early stages of the infection The key to the successful attainment of such a high viral recovery rate would appear to reside in the words properly collected stool specimens and early in the illness On the whole these stipulations can be carried out fairly well when the collection of clinical specimens is under the control of the investigator or of well indoctrinated collaborators However even such control does not necessarily lead at least in our experience to viral recovery rates greater than 75 to 80 per cent

The basis for requesting and examining multiple stool specimens from patients with suspected poliomyelitis is illustrated in Table 127 The results of virus isolation studies on serial stool specimens indicate (Table 128) that shedding of the virus in the feces is not necessarily a continuous process but apparently a discon

TABLE 127 RELATION BETWEEN INTERVALS PROPOSED FOR COLLECTION OF STOOL SPECIMENS AND INTERVALS ACTUALLY FOLLOWED BY PHYSICIANS SUBMITTING SPECIMENS

SPECIMEN	STOOL SPECIMENS REQUESTED		TOTAL NUMBER SPECIMENS	INTERVAL STOOL SPECIMENS COLLECTED		
				MEAN	MEDIAN	RANGE
	Prior to June 15 1955	After June 15 1955				
1st	Soon as possible after onset	When patient seen and 3 to 4 specimens taken at 3 to 4 day intervals during the early phase of the illness	87[illegible]	11 days	9 days	0–53 days
2nd	3 days postonset		758	17 days	15 days	2 73 days
3rd	6 days postonset		573	2[illegible] days	21 days	3 73 days
4th	10 days postonset		378	[illegible]7 days	27 days	5 73 days
5th	Not requested		113	27 days	27 days	13–43 days
6th	Not requested		55	30 days	30 days	21 44 days
7th	Not requested		38	31 days	35 days	27 51 days

TABLE 128 DISCONTINUOUS NATURE OF VIRUS EXCRETION IN POLIOMYELITIC PATIENTS

Name	Age	Sex	Clinical Status	Virus in Stool Type	Days After Onset of Illness 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	19	20	21
W M	11	M	Paralytic	1	+					+	+	−											
E C	8	F		1		+		−	+											−			
E D	32	F		1		+		−	−	−	−												
R K	1	M		1			+	+	−	−													
C A	2	M		1			+	+											+				
H R	7	M		1			−				+						−		−				
H D	11	F		3			−				−	+		−									
M C	1	F		1					−				+			+							
F B	24	F		1							−		−			+				−			
H J	8	M		1							−	+	+					+					
S C	2	F		1							+		+		+								
C W	32	M		1							+	+								−		+	
G B	1	F		1									−		−	+		+					
V L	7	M		1							−	−	−										+
W P	9	F		1																−	−		+
A C	4	F	Nonparalytic	1			+		+									+					
S B	6	M		1			−												+				
B G	31	F		1					+									+					
R F	2	M		1							−	−	+										
M M	6	F		1								−	−	−	−						+		

— = Stool tested negative for polio virus

\+ = Polio virus recovered from stool

tinuous or intermittent affair at least insofar as demonstrable amounts of virus are concerned As is shown in Table 128 in some patients the virus was present in every stool specimen examined whereas in other patients the virus was demonstrable in some specimens but not in others Obviously if the virus-isolation attempts had been restricted to the examination of single specimens the results in a number of cases might well have been negative

Data similar to those in Table 128 on the excretion pattern in poliomyelitic patients were obtained on a much larger series of patients than that presented for illustrative purposes in Table 128 Analysis of this larger body of data indicates that of the entire group of individuals shown to have been excreting virus at one time or another during the course of their disease only 75 to 80 per cent of the group excreted virus on any given day this *proportion of virus* shedders appeared to hold up to approximately 30 days postonset and seemed to decrease thereafter although the rate of decline cannot be estimated with accuracy because of the small number of individuals for whom stool specimens were available after this period These findings like those illustrated in Table 128 point to the desirability of testing multiple stool specimens and form the basis for a collection schedule according to which a stool specimen is obtained when the patient is first seen and at 3 to 4-day intervals thereafter during the early stages of the illness In the laboratory the specimens are tested two at a time in chronologic sequence that is the first 2 stools are tested simultaneously and if the results are negative the next 2 specimens are examined if these prove to be negative any additional specimens available are then examined when a virus is isolated the subsequent stool specimens are not examined

Examination of multiple stool specimens favors detection of virus excretion and the value of such a procedure for this purpose is shown in Table 129 In view of the data to be *presented in Table 130* it should be emphasized that Table 129 does not deal with a randomly selected cross section of the total patient population we have studied but represents a group of 231 patients on whom multiple stool specimens were available were tested and a poliomyelitis virus was recovered It will be noted that of the

TABLE 129 RELATION OF NUMBER OF SERIAL STOOL SPECIMENS TESTED TO DETECTION OF EXCRETION OF POLIOMYELITIS VIRUS

No. of Stool Specimens	No. of Patients	Number of Serial Stool Specimens that Were Examined Before Virus Excretion Was Detected: 1	2	3	4	5	6	7
				Number of Patients				
2	182	164	18					
3	24	18	3	3				
4	20	11	3	4	2			
5	4	3	0	0	0	1		
6	—	—	—	—	—	—	—	
7	1	0	0	0	0	1	0	0
Total	231	196	24	7	2	2	0	0
Per cent	100	84 8	10 4	3 0	9	9		
Cumulative per cent		84 8	95 2	98 2	99 1	100 0		

This table does not include 28 virus-shedding patients from whom only 1 stool specimen was [illegible] and 1 patient whose specimens were pooled.
Orphan virus isolates [illegible] not included.
If 2 stools were collected on the [illegible] day after [illegible], they were counted as a single specimen [illegible] did the results of virus isolation tests of the [illegible] specimens differ

group of 182 patients on whom 2 stool specimens were available the first specimen yielded a virus in 164 cases and examination of the second stool specimen yielded a virus in the 18 individuals in whom the first stool specimen was negative Similarly of the 24 individuals on whom 3 stool specimens were available the virus was recovered from the first stool specimen of 18 from the second stool specimen of 3 and from the third stool specimen of the remaining 3 individuals As is indicated at the bottom of the table the proportion of virus recoveries increased with the number of specimens examined Thus a poliomyelitis virus was recovered from the first stool specimen of 85 per cent of this group Examination of the second stool specimen led to the recovery of a virus from an additional 10 per cent to give a total virus isolation rate of 95 per cent Examination of 1 additional stool specimen from those individ

TABLE 130 VIRUS ISOLATIONS FROM PATIENTS WITH CLINICAL DIAGNOSIS OF POLIOMYELITIS

Virus Recovered	Proportion Virus Recoveries from Patients Diagnosed as: Paralytic Polio, Number	Paralytic Polio, Per cent	Nonparalytic Polio, Number	Nonparalytic Polio, Per cent	Total Patients, Number	Total Patients, Per cent
Polio Virus						
Type 1	173	49%	47	13%	220	31%
Type 2	4	1%	4	1%	8	1%
Type 3	29	8%	3	1%	32	4%
Types 1 and 2 and Coxsackie B 5 mixed	1	+%	—	—	1	+%
Other Virus						
Orphan	18	5%	102	28%	120	17%
Negative	187	37%	212	57%	339	47%
Totals	352	100%	368	100%	720	100%

Table includes all patients on whom laboratory work was complete as of March 15 1957
All 3 [illegible] from [illegible] stool specimen

uals whose first 2 specimens were negative increased the virus-recovery rate to 98 per cent and examination of a fourth specimen further increased the rate to 99 per cent. However the number of stool specimens to be examined must be considered not only in terms of what additional information is gained in relation to the time and the effort expended but also from the practical standpoint of how many specimens one can expect to be submitted Thus from Table 129 a virus-recovery rate of 98 per cent was associated with the examination of 3 serial stool specimens However considerable attrition occurs in the submission of multiple stool specimens and is progressively greater as the number of specimens increases

During the interval May 1955 at which time a restricted diagnostic service was initiated through March 15 1957 we have examined stool and blood specimens from a total of 720 patients on whom both types of specimens were submitted In most instances the stool specimens were tested in pairs as outlined above and the findings were presented in Table 130 (This table includes the results obtained with a number of stool specimens taken 30 or more days after onset some of these specimens proved to contain virus others did not.) Of the 720 individuals considered clinically to have or possibly to have poliomyelitis the diagnosis in 352 cases was paralytic poliomyelitis and in 368 it was nonparalytic poliomyelitis either suspected or definitely stated

Of the 352 patients with a clinical diagnosis of paralytic poliomyelitis a poliomyelitis virus was recovered from only 206 individuals or 58 per cent of the group If a poliomyelitis virus-recovery rate of 90 per cent is accepted as an attainable goal then our virus-recovery rate is only two thirds of what it presumably could or should be Whether or not our comparatively low isolation rate can be attributed to failure to collect stool specimens sufficiently early in the illness to improper methods of collection and handling and to shipment without refrigeration remains to be determined However we believe that it is an accurate reflection of what can be expected in the everyday practice of diagnostic virology as applied to clinical medicine Confronted with the realities of the situation it is obvious that adjunct diagnostic measures are required and such measures, namely serologic technics will be considered in a moment.

With respect to the 368 individuals in whom a diagnosis of nonparalytic poliomyelitis was entertained clinically a poliomyelitis virus was recovered from the stools of only 54 individuals, or 15 per cent of the group Proportionally this

TABLE 131 NONPOLIOMYELITIS VIRUSES ISOLATED FROM PATIENTS CLINICALLY DIAGNOSED OR SUSPECTED TO HAVE POLIOMYELITIS

CLINICAL IMPRESSION	NUMBER OF PATIENTS	NUMBER OF PATIENTS FROM WHOM VIRUS RECOVERED WAS: COXSACKIE					ECHO			NOT YET IDENTIFIED
		A 9	B 2	B-3	B-4	B-5	4	6	7&8	
Paralytic polio	19	1	3		1	2		1		11†
Nonparalytic polio	84	7	4	3	3	25‡	1		1§	40
Suspect polio	1									1
Polio vs Coxsackie	3					2				1
Polio vs encephalitis	8					5				3
Encephalitis	4					3				1
Pleurodynia(?)	1					1				
Diagnosis deferred	1									1
Totals	121	8	7	3	4	38	1	1	1	58

These are the 121 patients shown in Table 130 under Orphan" virus and mixed "
Type 1 and 2 virus also isolated from the stool of 1 of these patients.
† Type 1 virus also isolated from stool of 1 patient.
One patient had serologic evidence of Type 3 polio virus infection
§ In this patient, ECHO 7 virus was isolated from 1 stool ECHO 8 from another stool
^ Five patients had serologic evidence of polio (2 Type 1 2 Type 2 and 1 Type 3)

nonparalytic group yielded more "orphan viruses than did the group considered clinically to have paralysis since 107 enteric cytopathogenic viruses other than poliomyelitis were recovered from the nonparalytic group and 19 such viruses were recovered from the paralytic group an incidence of 28 per cent and 5 per cent respectively The 171 patients who yielded an orphan virus (Table 131) represent 17 per cent of the total group of 720 patients this is a fairly impressive figure numerically and it becomes more impressive when one considers the time and the effort that the identification and the typing of these agents demand if they happen to be members of the Coxsackie or ECHO families The present neutralization tests in monolayer cell cultures are cumbersome not inexpensive and leave something to be desired A simpler method for the identification of the Coxsackie and the ECHO groups of viruses for diagnostic purposes is highly desirable The possibility of typing these agents by means of a reverse complement fixation test as for example can be done for identification of Western equine encephalomyelitis virus or by a colorimetric neutralization test as has been described recently for the adenoviruses merits investigation

DIAGNOSIS BY MEANS OF NEUTRALIZATION OR COMPLEMENT FIXATION TESTS

For serologic purposes 2 or more serial blood specimens are requested on each patient The specimens are always examined simultaneously in the same test The neutralizing antibody content of the sera is determined by the method of Salk *et al* using a monkey kidney cell system or by the method of Syverton *et al* or of Lipton and Steigman using HeLa or KB cell systems Poliomyelitis complement fixation tests are performed according to the macroscopic (tube) technic described in recent publications from this laboratory The demonstration of a fourfold or greater rise in antibody titer by either of these methods generally is accepted as diagnostically significant As is true in general of all viral diagnostic work the ability to detect a significant rise in titer depends on a proper temporal spacing of the serum specimens in relation to the disease process The first serum specimen should be taken as promptly after the onset of the illness as possible (Table 132) As concerns poliomyelitis many patients already possess maximal levels of neutralizing or complement fixing antibody by the time that they are admitted to the hospital or by the time that poliomyelitis is suspected Neutralizing antibody titers especially are apt to be at maximal levels On the other hand the later the acute phase blood specimen is taken the greater the likelihood that antibody will be present and if there is sufficient delay maximal antibody titers will have been reached in the latter case it obviously will be impossible to detect rises in antibody levels and it will be equally difficult or impossible especially in the case of neutralizing antibody to interpret unequivocally the significance of the antibody which is present

The value of a serologic test is not only a matter of its sensitivity in the detection of specific antibody but also a matter of the extent to which it can reveal the occurrence of diagnostically meaningful rises in antibody levels To evaluate the practical usefulness of a test it is necessary to examine paired or multiple serum specimens from patients known to be infected and to ascertain the proportion of cases in which

TABLE 132 RELATION BETWEEN INTERVALS PROPOSED FOR COLLECTION OF BLOOD SPECIMENS AND INTERVALS ACTUALLY FOLLOWED BY PHYSICIANS SUBMITTING SPECIMENS ON 306 PATIENTS

Specimen	Blood Specimens Requested	Total Specimens	Interval Blood Specimens Collected Mean	Median	Range
1 Acute phase	As soon as possible after onset	306	7 days	5 days	0–30 days
2 1st conv	14 days postonset	275	25 days	23 days	10–60 + days
3 2nd conv	28–40 days postonset	103	31 days	30 days	17–60 + days†

O pec men t ke m r th n 60 d aft r o set
† F r pec me t k n m e than 60 d ys afte on et

TABLE 133 DISTRIBUTION BY DAYS AFTER ONSET OF HOMOTYPIC NEUTRALIZING ANTIBODY TITERS OF 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

Homotypic Neutralizing Antibody Titer	Days Between Onset of Illness and Collection of Specimens								
	0–5	6–10	11–20	21–30	31–40	41–50	51–60	61–	Total
1:2 048			2						2
1:1 024	3	5	4	8	1	3		1	25
1:512	5	8	3	4	4	5	5	1	35
1:256	14	13	8	11	6	2	5		59
1:128	14	5	9	22	2	1	2		55
1:64	12	13	2	6		1			34
1:32	12	6	1		1				20
1:16	5	1							6
1:8	4	3							7
1:4		1							1
<1:4	2	2							4
Total	71	57	29	51	14	12	12	2	248

increases in antibody titer are detectable. Paired blood specimens from 124 patients from whom a poliomyelitis virus had been recovered were examined for their neutralizing and complement fixing antibody levels. The distribution of the neutralizing antibody titers in the acute and the convalescent phase blood specimens is shown by days after onset in Table 133.

It will be noted that the low antibody titers present from 0 to 10 days postonset were converted to higher levels by 11 or more days post onset so that by the 11th day in only 2 individuals were neutralizing antibody titers of less than 1:64 encountered. It also will be noted that a rather large proportion of individuals possessed antibody titers of 1:64 or greater within the interval 0 to 5 days and 0 to 10 days postonset; that is, quite high and even maximal antibody levels were encountered shortly after the onset of illness.

TABLE 134 DISTRIBUTION BY DAYS AFTER ONSET OF HOMOTYPIC COMPLEMENT FIXING ANTIBODY TITERS OF 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

Homotypic C F Antibody Titer	Days Between Onset of Illness and Collection of Specimens								
	0–5	6–10	11–20	21–30	31–40	41–50	51–60	61–	Total
1:128							1		1
1:64	2	5	11	5		2	2		27
1:32	5	5	8	15	8	5	2	1	49
1:16	5	4	4	18	4	2	5		42
1:8	5	9	3	6	1	2	1	1	28
1:4	13	7	3	7	1	1	1		33
<1:4	41	27							
Total	71	57	29	51	14	12	12	2	248

This is in contrast with the findings presented in Table 134 which shows the distribution of complement fixing antibody titers according to the interval after onset of illness at which the blood specimen was collected It will be noted that as in the case of neutralizing antibody there was a trend toward higher antibody titers with increasing time postonset but unlike the situation with neutralizing antibody the number of individuals with high titers shortly after onset was quite small Thus taking the interval 0 to 10 days postonset 68 serum specimens had complement fixing antibody titers of less than 1 4 as compared with only 4 sera with neutralizing antibody titers of less than 1 4

Tables 133 and 134 illustrate in an approximate fashion because of the relatively small numbers the distribution of antibody levels that may be expected at given intervals postonset of illness To ascertain the ability of the neutralization and the complement fixation tests to reveal increases in antibody concentration during the course of the illness it is necessary to compare the number of instances in which a significant rise in antibody titer was demonstrable Such a comparison is made in Table 135 in which the rises in homotypic antibody (antibody corresponding to the virus type recovered from the stool) as determined by examination of paired sera are compared As is shown in the upper left hand portion of Table 135 no diagnostically significant change in titer occurred in 22 individuals or 18 per cent of the 124 patients in the group On the basis that a fourfold or greater rise in antibody level constitutes a diagnostically significant increase 21 patients or 17 per cent of the group showed a rise in neutralizing antibody but not in complement fixing antibody This is in contrast with the finding that more than twice as many individuals showed a rise in complement fixing antibody but not in neutralizing antibody this occurred in 45 individuals or 36 per cent of the group In 36 patients or 29 per cent of the total number of individuals a fourfold or greater rise in both neutralizing and complement fixing antibody occurred If these figures are accumulated it will be found that 81 individuals or 65 3 per cent of the test group showed a significant rise in complement fixing antibody whereas only 57 individuals or 46 per cent of the entire group showed a diagnostically significant rise in neutralizing antibody (These figures are of interest because of their close correspondence to those

TABLE 135 COMPARATIVE VALUE OF THE COLORIMETRIC NEUTRALIZATION TEST AND THE COMPLEMENT FIXATION TEST IN DEMONSTRATING DIAGNOSTICALLY SIGNIFICANT CHANGES IN ANTIBODY LEVEL PAIRED SERA FROM 124 PATIENTS WHO EXCRETED VIRUS IN STOOLS

CHANGE IN HOMOTYPIC NEUTRALIZING ANTIBODY TITER	CHANGE IN HOMOTYPIC COMPLEMENT FIXATION TITER −2X	0	+2X	+4X	+8X	+16X	+32X	+64X	TOTAL
4X					1				1
−2X		1	2	2	1				6
0	1	5	6	6	6	4	1		29
+2X			7	10	5	4	4	1	31
+4X	1	2	9	2	7	2	2		25
+8X	2	1	2	2	4	6	1		18
+16X			2	2	1	2	2		9
+32X									—
+64X			2			1			3
+128X					1		1		2
Total	4	9	30	24	26	19	11	1	124

4X o G at r Tt R
CF 81/124 = 65 3
N t 57/124 = 46 0
CF Neut 102/124 = 82 3

obtained in another study concerned with the development and the persistence of neutralizing antibody In that study a rise in homotypic complement fixing antibody was encountered in 66 per cent of the patients comprising the study group and a rise in homotypic neutralizing antibody was encountered in 42 per cent of the patients) If we combine the groups in which rises in complement fixing or neutralizing antibody or both occurred we find that a rise in the titer of either antibody was demonstrable in 102 individuals or 82 per cent of the group studied

The complement fixation test because of its simplicity and the rapidity with which results can be obtained possesses definite advantages over the neutralization test From our experience it appears to possess the additional merit of detecting antibody rises in a greater proportion of instances than does the neutralization test *an advantage which is referable to the* comparatively slow appearances and development of complement fixing antibody By performing both complement fixation and neutralization tests on the same specimens the proportion of patients in whom diagnostically significant antibody rises could be detected was increased from 65 per cent to 8? per cent that is by approximately one fourth Whether or not this added advantage is worth the large amount of additional labor involved in such dual serologic examinations has yet to be determined

Let us now consider the application of serologic tests to the diagnosis of illness in patients from whom no virus was recovered Reference to Table 130 shows that an attempt to reach an early diagnosis through the examination of stool specimens was made in 720 patients Of this group 352 patients were considered clinically to have the paralytic disease and 368 were considered or suspected to have the nonparalytic form No virus was recovered from 177 patients in the former group nor from 212 patients in the latter group

Paired or multiple blood specimens on each of the 339 patients in this virus negative group were examined to determine whether or not rises in titer of antibody had occurred to any one or more of a number of viruses Thus the

TABLE 136 RESULTS OF SEROLOGIC TESTS ON 339 PATIENTS WITH CLINICAL DIAGNOSIS OF POLIOMYELITIS BUT FROM WHOM NO VIRUS WAS ISOLATED

Serologic Diagnosis	Proportion of Patients with Indicated Laboratory Diagnosis Who Were Clinically Regarded As				Total Patients	
	Paralytic Polio		Nonparalytic Polio			
	Number	Per cent	Number	Per cent	Number	Per cent
Poliomyeliti						
Type 1	37	29%	33	16%	70	21%
Type 2	7	6%	22	10%	29	9%
Type 3	16	13%	19	9%	35	10%
Other Virus						
Mumps	12	9%	30	14%	42	12%
Herpes simplex	1	1%	1	1% (Herpes simplex, WEE, SLE)	2	1% (Herpes simplex, WEE, SLE)
WEE*	—	—	1		1	
SLE†	1	1%	1		2	
Dual Infection	3‡	2%	1§	+%	4	1%
Diagnosis Uncertain	12	9%	14	7%	26	8%
Negative	38	30%	90	43%	128	38%
Total	127	100%	212	100%	339	100%

Tabl includes all pat ents on wh m l bor tory wo k wa complete as of M rch 15 1957
* Western equ ne ncephal ti
† St. Louis en eph liti
‡ Includes 1 ase infl nza plu Typ 1 pol o 1 c se i flu nza plus Type 2 p l o nd 1 ca Type 1 plus Type 2 polio
§ Type 1 pol o plu mumps virus

sera were examined for both neutralizing and complement fixing antibody levels to poliomyelitis virus and for complement fixing antibody to herpes simplex mumps Western equine and St Louis encephalitis viruses and occasionally other viruses as indicated The results of the serologic tests are reported in Table 136

Of the 127 individuals with a clinical diagnosis of paralytic poliomyelitis 60 or 48 per cent showed a diagnostically significant rise in poliomyelitis antibodies 12 individuals showed a rise in mumps antibodies 1 a rise in herpes simplex antibodies and 1 a rise in St Louis encephalitis antibodies Three instances of dual infection were encountered namely 2 cases of influenza and poliomyelitis and 1 case of dual infection with 2 poliomyelitis viruses (The last case mentioned may represent an instance of heterotypic antibody response but is considered here to be a dual infection because of the very marked rise in antibody titer to 2 virus types) In 12 patients the significance of the serologic findings could not be interpreted unequivocally and these cases are listed as diagnosis uncertain In the remaining 38 individuals or 30 per cent of the group the results of all serologic tests were negative

Of the 212 patients in whom nonparalytic poliomyelitis was suspected or on whom such a diagnosis had been made or was entertained 74 or 35 per cent gave serologic evidence of poliomyelitis In 30 patients or 14 per cent of the group a rise in mumps virus antibody was observed and in 3 others the laboratory diagnosis was herpes simplex Western equine encephalitis or St Louis encephalitis virus infection 1 patient gave evidence of a dual infection with poliomyelitis and mumps viruses In 14 patients the serologic findings were inconclusive so that a clear-cut diagnosis was not possible and in 90 others the serologic findings were negative

SUMMARY

In this discussion I have tried to present some of the problems encountered by a virus laboratory in connection with diagnostic work on poliomyelitis and to show how we have attempted to meet some of these problems and how certain other problems might perhaps be attacked or resolved

Excretion of poliomyelitis virus in the stool appears to be intermittent and consequently the probability of recovering a virus is considerably enhanced if a series of stools is examined Despite this our virus recovery rate from patients suspected to have or definitely considered to have poliomyelitis has been 58 per cent in the paralytic group and 15 per cent in the nonparalytic group We raise the question whether or not the proportion of recoveries of the poliomyelitis the Coxsackie and the ECHO viruses might not be increased through replacement of the widely employed monkey kidney cell cultures by conceivably a cell type with a broader spectrum of viral susceptibility or less ideally by several cell types which conjointly afford a continuum of viral susceptibility It is possible also that the high percentage of negative results might be reduced to some extent through the use of 1-day-old mice in conjunction with tissue culture systems for virus isolation purposes

We believe that virus isolation since this affords a means of reaching an early diagnosis should be attempted on every patient with suspected poliomyelitis on whom laboratory diagnostic assistance is desired The significance of any agent isolated must be evaluated in terms of the patient s clinical history and course and his epidemiologic background Failure to isolate the virus does not necessarily negate a clinical diagnosis other laboratory approaches must be utilized Not infrequently the diagnosis of poliomyelitis depends on serologic tests and of the 2 procedures most widely tried thus far the complement fixation test appears to be preferable to the neutralization test because of the higher proportion of cases in which diagnostically significant rises in antibody can be detected Furthermore widespread artificial immunization against poliomyelitis threatens to impair seriously the usefulness of the neutralization test and even may affect adversely the complement fixation test to an as yet unknown extent

Serologic tests for viral agents known to be capable of producing CNS disturbances should be included in the examination of sera from patients suspected to have poliomyelitis Such a battery of tests is most useful when poliomyelitis virus recovery attempts as well as the poliomyelitis serology are negative and should

include in addition to tests for the mumps virus tests for those viruses known to produce CNS disease in the area or region within which the diagnostic laboratory operates

ACKNOWLEDGMENTS

Many members of the staff of the Viral and Rickettsial Disease Laboratory participated in the work which forms the basis of this paper and I wish to express my thanks and appreciation to each I am especially indebted to Miss Anna Wiener in charge of the poliomyelitis virus isolation unit to Mr Tak Shinomoto for the colorimetric neutralization tests and to Dr Nathalie Schmidt for the complement fixation tests

My thanks also are extended to Mrs Antonietta Cincotta for liaison work with patients and physicians and supervision of the clerical aspects and to Mr Ray Guenther for tabulation and collection of much of the data

BIBLIOGRAPHY

Godenne M O and Riordan J T Tissue culture diagnosis of poliomyelitis and aseptic meningitis J A M A 158 707 1955

Kilbrick S Enders J F and Robbins F C An evaluation of the roller tube tissue culture for the isolation of poliomyelitis viruses from feces J Immunol 75 391 1955

Lennette E H Neff B J and Fox V L A colorimetric method for the typing of adenoviruses Am J Hyg 65 94 1957

Lennette E H and Schmidt N J Studies on the development and persistence of complement fixing and neutralizing antibodies in human poliomyelitis Am J Hyg 65 210 1957

Lennette E H Wiener A Ota M I Fujimoto F Y and Hoffman M N Rapid identification of isolates of Western equine encephalomyelitis virus by the complement fixation technique Am J Hyg 64 270 1956

Lipton M M and Steigman A J A simplified colorimetric test for poliomyelitis virus and antibody Proc Soc Exper Biol & Med 88 114 1955

Melnick J L Isolation of virus and the development of neutralizing and complement fixing antibodies in poliomyelitic patients Ann New York Acad Sc 61 1005 1955

Nolan J P Wilmer B H and Melnick J L Poliomyelitis its highly invasive nature and narrow stream of infection in a community of high socioeconomic level New England J Med 253 945 1955

Paul J R and Melnick J L Poliomyelitis in Francis T Jr (ed) Diagnostic Procedures for Virus and Rickettsial Diseases ed 2 pp 53 90 New York Am Public Health A 1956

Salk J E Youngner J S and Ward E N Use of color change of phenol red as the indicator in titrating poliomyelitis virus or its antibody in a tissue culture system Am J Hyg 60 214 1954

Schmidt N J and Lennette E H A complement fixation test for poliomyelitis J Exper Med 102 133 1955

—— Modification of the homotypic specificity of poliomyelitis complement fixing antigens by heat J Exper Med 104 99 1956

Schmidt N J Lennette E H Doleman J H and Hagens S J Factors influencing the potency of poliomyelitis complement fixing antigens produced in tissue culture systems Am J Hyg 66 1 1957

Discussion—Isolation of Viruses

DR ALBERT B SABIN

During the acute phase of infection isolation of virus is the most useful procedure for establishing a diagnosis of poliomyelitis Since Dr Lennette reports failure to find virus in 25 per cent of a group of 65 paralytic patients selected as typical cases of poliomyelitis when the stools were collected early after onset and an even lower incidence of isolations in other groups one is led to wonder especially about such factors as actual time after onset that his specimens were collected the clinical diagnostic problems and the possible inactivation of virus during shipment at specially high summer temperatures Studies on human beings experimentally infected with attenuated strains have shown that some persons may excrete virus for only 7 to 10 days after ingestion so that with an incubation period of this duration one could expect a certain proportion of patients not to have demonstrable virus even early after onset Such studies have also shown that the amount of virus excreted per gram of stool diminishes with time and the intermittent excretion mentioned by Dr Lennette is an indication that one is dealing with the end stage of viral multiplication During the early stages of clinically recognized or inapparent infection 1 Gm of stool may contain more than 1 000 000 tissue-culture infective doses of virus and on several occasions we have recovered 100 000 to 800 000 infective doses from a single rectal swab

In our own hospital and laboratory polio virus was isolated in 100 per cent of a consecutive series of 24 patients with a clinical diagnosis of paralytic poliomyelitis from whom stool or enema specimens were obtained within 2 to 8 days after onset of first symptoms—this being dated from the first relevant complaint observed by the patient or the mother In the same group of patients we found that a single throat swab obtained 2 to 6 days after onset of first symptoms yielded polio virus in 83 per cent A dry cotton swab was passed over the posterior pharyngeal wall several times and put into 2 ml of Hanks s solution containing 2 000 units penicillin 2 mg streptomycin and 250 units mycostatin per ml The swab was rubbed along the sides of the tube the fluid was squeezed out and the material was ready for use The results of the tests in monkey kidney cultures shown in Table 137 indicate that the incidence of positive isolations was about the same 4 5 and 6 days after onset as at 2 and 3 days but that it was down to 50 per cent at 7 and 8 days 5 of these 29 patients had a nonparalytic infection with polio virus in their stools The results shown in Table 138 indicate the number of infective doses in each individual swab which ranged from 6 to 3? 000 The zeros mean that virus was not found in the 0 6 ml of fluid that was tested and it is possible that if the entire

TABLE 137 EFFICACY OF THROAT SWABS FOR DIAGNOSIS OF POLIOMYELITIS DURING FIRST 8 DAYS AFTER ONSET OF SYMPTOMS

DAYS AFTER ONSET	NO OF PATIENTS TESTED	NO POSITIVE	PERCENTAGE	
2 3	10	8	80	19/23 = 83%
4 5 6	13	11	85	
7 8	6	3	50	
Totals	29	22	76	

21 w Type 1 1 w Type 3 nly 2/29 p ts w Type 3

TABLE 138 AMOUNT OF VIRUS PER THROAT SWAB IN INDIVIDUAL PATIENTS AT DIFFERENT TIMES AFTER ONSET OF SYMPTOMS

DAYS AFTER ONSET OF SYMPTOMS	TITER OF VIRUS IN INDIVIDUAL SWABS LOG TO BASE 10						
2	4 5	3 0	3 0	1 5	1 5	0 0*	
3	4 0	2 0	1 3	0 0	—	—	
4	4 0	1 5	0 0	—	—	—	
5	4 5	4 0	2 5	2 5	2 0	1 5	0
6	2 5	2 0	2 0	—	—	—	
7	1 3	0 0	0 0	0 0	—	—	
8	2 0	0 8	—	—	—	—	

0 = None found in 0 6 ml

2 ml of swab fluid had been tested or if more than one swab had been used the incidence of positive isolations might have been still higher The procedure of culturing polio virus directly from a throat swab or a rectal swab is so simple and the specimens are so easily obtained on admission of the patient that tests on stools could be limited only to those patients whose throat and rectal swabs fail to yield virus

Virus isolation becomes increasingly unreliable as a diagnostic procedure as the interval after onset of infection becomes longer and in such cases the serologic tests may provide the only means of establishing the diagnosis While the etiologic role of certain Coxsackie and ECHO viruses in the aseptic meningitis syndrome is now well established there have been instances in which patients exhibited serologic evidence of infection with polio virus while the stools yielded only a Coxsackie or an ECHO virus Accordingly failure to isolate polio virus from a patient exhibiting a clinical syndrome compatible with poliomyelitis does not rule out the diagnosis unless appropriate serologic tests can be interpreted as indicating that no recent infection with polio virus has occurred

Discussion—Neutralization Tests

DR JONAS E SALK

Dr Smith indicated that each of us is interested in another aspect of the problem of diagnosis Our interest is in the diagnosis of immunity in poliomyelitis rather than in the diagnosis of disease Dr Lennette has so adequately discussed the neutralization test in relation to diagnosis of disease that I will restrict my remarks for the most part to the diagnosis of immunity I will not go into detail in regard to the different technics whether they be the plaque test of Dulbecco the use of roller tubes or the color test

First let me show you in a few slides the results of a test for immunity In Figure 230 are shown data obtained in a study involving

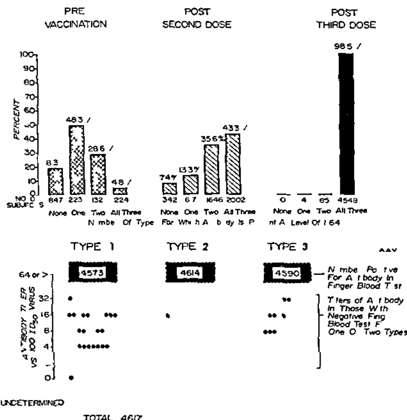


FIG 230 (*Top*) Per cent distribution of individuals with antibody titers of 1 64 or greater before and after vaccination (4 617 subjects given 2 doses 2 weeks apart third dose 1 year later)

FIG 231 (*Bottom*) Antibody levels determined on samples of venous blood after 3 doses of vaccine in those who gave negative finger blood test for antibody at level of 1 64

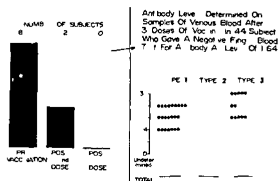


Fig 232 Number of subjects with no antibody for all 3 types at level of 1 64

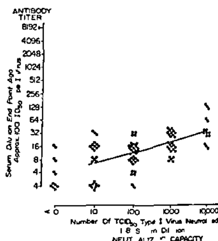


Fig 234 Correlation of two metho serum antibody measurement neutrali capacity vs antibody titer (constant se vs constant virus) serum samples fr mixed group of persons after one or r doses of vaccine

4 617 individuals in whom the blood tests were done on a drop (0? cc) taken from the finger whereby a test could be made of a 1 64 dilution of serum before or after vaccination You will observe that at the 1 64 level only 4 8 per cent individuals had antibody to all 3 types and 18 3 per cent had no antibody to any type at that level After the second dose of vaccine you will see an upward shift in the distribution of those with antibody then following the third dose given about a year later you will observe that 98 5 per cent of individuals had antibody to all 3 types at a level of 1 64 or greater

We were then interested in the 1 5 per cent that did not have demonstrable antibody at the 1 64 level in the finger blood test Venous blood was obtained and you will see (Fig 231) that in all but one instance antibody for all 3 types was present at lower levels of serum dilution When an analysis was made merely of the 847 individuals who had no antibody to any 3 types those who developed antibody 3 types after the second or third dose a cated the 44 who had no antibody to all at the 1 64 level in the finger blood tes examined and you will see the results right hand portion of Figure 232

I want to show an anomaly to indica haps the desirability of having a test for nity Figure 233 left depicts a child who received the inoculations at the inter months indicated You will see the 1 and 2 response but there seemed to be

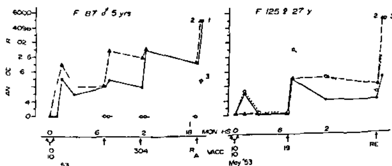


Fig 233 Response variation among individuals to the 3 antigens

toriness to Type 3 until an inoculation given some 18 months later Other individuals simi larly treated responded more adequately Fig ure 733 right shows another anomaly of a poor Type 1 response with a good Type 2 and 3 this too was anomalous when compared with the group as a whole

The data in Figure 234 indicate the results of the two ways in which a test for antibody can be made and raise the question as to what is meant by the number of antibody units expressed by antibody titer as indicated on the vertical scale Antibody measurements on the horizontal scale shown were tested using a 1 8 serum dilu tion and determining its neutralizing capacity against different dilutions of virus You will observe that in some instances serum from indi viduals who had what appeared to be relatively low levels of antibody by the serum-dilution method shown on the vertical scale had the capacity to neutralize large amounts of virus as indicated on the horizontal scale

The significance and the importance of this may be that perhaps we may not be measuring the significant antibody status of an individual when we measure the dilutions of serum against a constant amount of virus What we really want to know in a diagnosis for immunity is how much virus will be neutralized by the indi vidual s undiluted serum Therefore a test such as the one in which perhaps a 1 8 dilution of serum is used and is tested against different dilutions of virus may give us a better measure of immunity and of immune status moreover technically it is far simpler to perform

In Figure 235 is something that is purely theoretical The word valence should have been in quotation marks I have no way of knowing whether we are talking about valence avidity or some other factor but when we plotted the observed relationship between neu tralizing capacity of a 1 8 dilution of serum against the serum dilution end point from the data in the previous slide a line of gradual slope was observed If the virus increments and the antibody differences were approximately equally related one would have expected a slope of the order of magnitude shown in the right hand insert Therefore under the circumstances we wonder whether or not different intensities of immunization as have been observed for other immunizing agents do not alter the qual ity of the antibody either by way of avidity or valence so as to provide a better measure of actual immunizing effect of the serum by testing a constant dilution of serum against different dilutions of virus rather than different dilutions of serum against a constant dilution of virus

These are random thoughts on this subject which is being studied more intensively at the present time

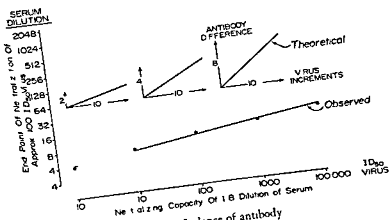


Fig 235 Valence of antibody

Discussion—Precipitin Test

DR G L LE BOUVIER

Precipitates can be formed by the reaction between antigens present in polio virus concentrates and antibodies present in the serum of patients convalescent from poliomyelitis or of monkeys hyperimmunized against polio virus These precipitates are type specific They may be observed in tests using liquid reactants alone or they may be examined by one or another of the gel-diffusion methods I shall discuss some results obtained by the method of Ouchterlony in which the virus and serum reactants diffuse from separate cups into an agar gel contained in a Petri dish

Monkey antisera may produce two bands of precipitate in the agar comprising a minor as well as a major band but human sera in the tests so far performed have produced only the major band and it is this which is referred to here The point between the virus and the serum cups at which a precipitate develops is a measure of the relative concentration of the homotypic antigen and antibody concerned This fact makes it possible to standardize the conditions of the test and measure the polio virus precipitin content of a specimen of serum *

Figure 236 shows the appearance after 5 days of incubation at 37 C of a precipitin test on paired sera from 2 patients with poliomyelitis (a b and a b) These sera are undiluted The center cup contains Type 3 polio virus consisting of the liquid from infected monkey kidney cultures concentrated 50 times by ultracentrifugation On either side are cups containing different dilutions of a type specific reference serum The precipitate formed by the higher concentration of reference serum (1/4) is further away from the serum cup and closer to the central virus cup than that formed by the lower concentration (1/16) Both patients had a Type 1 virus infection one of them whose sera (a b) are seen in the upper half of the picture, possessed no detectable Type 3 precipitins the other had about the same amount of antibody in both specimens (a′ b) as is shown by the similar position and the appearance of the precipitates The fusion of these precipitates with those produced by the reference serum argues the identity of the antigen antibody systems involved

Using unheated crude virus concentrates, polio virus precipitins were assayed in 60 specimens of sera These consisted of acute and convalescent phase sera from 21 patients with either Type 1 or Type 3 virus infections and of serum collected from 9 people before and 1 month after the feeding of attenuated Type 1 polio virus

In Figure 237 the precipitin content of each one of these sera is compared with its titer of neutralizing antibody against the same virus type namely the one causing the infection Each of the points represents 1 serum specimen (The different appearances of the individual points may be disregarded in the present context) There seems to be a general tendency for polio virus precipitating antibodies and the homotypic neutralizing antibodies to run parallel at least during the first 2 months after the onset of illness It should be mentioned that these are total neutralizing antibodies determined by the pH test in panels and would include those described as being of low avidity in addition to the so-called high avidity" neutralizing antibodies A correlation has also been found during the first 2 months after infection between the precipitins detectable with crude virus concentrates and the kind of complement fixing antibodies reacting with unheated as distinct from heated polio virus

From these findings it would appear that there is no *special* information to be gained from the investigation of polio virus precipitins

* In case tests for polio virus precipitins should come to be tried out in different laboratories it may be well at this stage to enter a plea that standard reference reactants be designated and used from the start so that all such tests wherever performed may be capable of being compared

or at any rate none that would set them apart from the neutralizing and the complement fixing antibodies Therefore even supposing that adequate supplies of virus concentrates were to become available it seems unlikely that precipitin tests on paired sera would be adopted widely as a regular diagnostic procedure If precipitin tests do possess any peculiar advantage it may lie in the speed with which they can provide an indication of the serum antibody content in the first days or even hours of illness For such a purpose the sort of test described here would be less suitable than either the far swifter tube flocculation test about which Dr Churcher is going to speak or the beautifully neat and ingenious application of the agar-diffusion method about which I believe our esteemed General Chairman of the Conference will have something to say

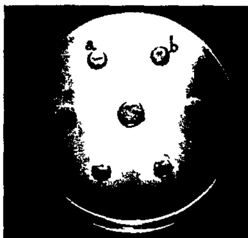


Fig 236 Appearance of a precipitin test on paired sera from 2 patients with poliomyelitis after 5 days of incubation at 37 C

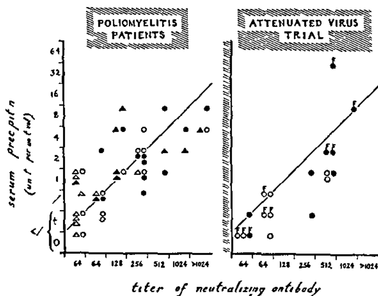


Fig 237 Precipitin content of each serum compared with its titer of neutralizing antibody (each point represents 1 serum specimen)

Discussion—Complement-Fixation Tests

DR JOSEPH L MELNICK

Dr Lennette has adequately described the application of the macrotube complement fixation (CF) test for the diagnosis of poliomyelitis The Fulton and Dumbell plate technic also may be used and has the advantage of requiring smaller quantities of reagents In the latter method several different concentrations of complement are used with each dilution of antigen or serum in order (1) to determine the amounts of complement fixed and (2) to give added sensitivity by a quantitative estimation of and compensation for anticomplementary factors in the reagents However the use of the plate technic does not necessitate the use of multiple levels of complement and it may be used for 1-dimensional serum titrations similar to those described for the tube method Both the tube and plate methods have recently been outlined in the second edition of ***Diagnostic Procedures for Virus and Rickettsial Diseases*** published by the American Public Health Association

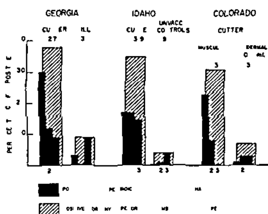


Fig 238 Percentage of persons givin positive complement fixing response t Type 1 2 or 3 or to any type or combina tion of types of polio virus after 1 inocula tion with Cutter ne Controls were either unva ons in the same area or thos ith a vac cine other than th tter

Dr Lennette has described in detail the application of the complement fixation test but a few general remarks about this test in poliomyelitis seem to be in order at a conference such as this A strong positive complement fixation reaction may be interpreted as evidence of probable recent experience with polio virus The complement fixation antibody response occurring in individuals who have had prior experience with polio virus of any type is usually prompt and often group reactive Therefore it is often difficult to demonstrate an increase or to identify the type of the current infection in these individuals However their early response to *heated* antigens allows one to make a tentative diagnosis in these cases on the basis of the first serum sample alone

There appear to be 2 kinds of complement fixation antibodies formed after infection with polio virus particularly in older children and in adults who presumably have had earlier experience with one or more types of polio virus

1 Antibodies against the group antigens are made rapidly and generally have reached their highest titer by the onset of illness although in some patients they make their appearance soon after onset and increase in titer over the next few weeks Exposure of polio virus to heat (or ultraviolet irradiation or formalin) uncovers a previously masked group-reactive antigen common to the 3 virus types which is suitable for detecting this group antibody By using heated antigens heterotypic fixation (against polio virus types different t isolated *from the*
ient) may occu absence of corre
ding heterotyp ing antibodies
Antibodies ag specific antigen
ted with th re usually absent
acute phas us appear
r the thr ple
ion antil ific
is gen the
eutra the

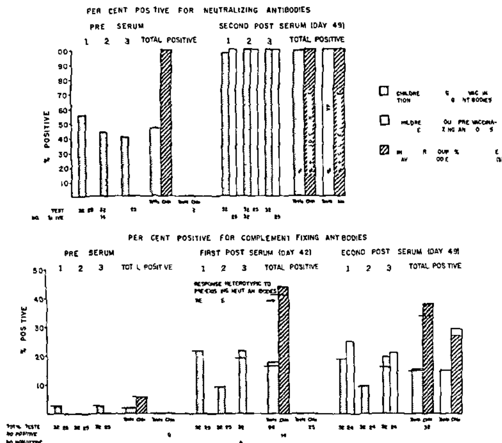


FIG 239 Neutralizing and complement fixing antibody responses to 3 inoculations with Vaccine P by 57 children whose average age was slightly under 3 years Responses to each type of polio virus are shown by dotted columns for the children who had neutralizing antibodies to some type before vaccination and by white columns for the children who had no neutralizing antibodies to any type before vaccination The cross-hatched columns show the per cent of children having antibodies to any type in the serum taken on the day indicated

Our recent experiments have shown that the complement fixation antigen unit is only 8 mμ in diameter in contrast with the diameter of the infective particle which is 27 mμ in diameter These measurements were obtained as mentioned earlier at this conference by subjecting polio virus to ionizing radiation and determining the relative rates of inactivation of infective virus and the complement fixation antigen The data suggest that within the virus particle there exist several complement fixation antigenic units

To date the response with the complement fixation antibody response in *vaccinated children* suggest that both dead and live viruses can elicit complement fixation responses but the level of response after infection appears to be much greater than after vaccination with killed virus First I would like to show the data obtained in the spring of 1953 when live virus

NEUTRALIZING AND COMPLEMENT FIXING ANTIBODY RESPONSE TO VACCINE Q

PER CENT POSITIVE FOR NEUTRALIZING ANTIBODIES

PER CENT POSITIVE FOR COMPLEMENT FIXING ANTIBODIES

Fig 240 Neutralizing and complement fixing antibody responses to 2 inoculations with Vaccine Q by 78 children whose average age was slightly under 6 years Responses to each type of polio virus are shown by dotted columns for the children who had neutralizing antibodies to some type before vaccination and by white columns for the children who had no neutralizing antibodies to any type before vaccination The cross hatched columns show the per cent of children having antibodies to any type in the serum taken on the day indicated

inadvertently slipped through the inactivation process and was used in the field The Cutter material contained traces of live virus With the aid of the Epidemiology Branch of the Communicable Disease Center we carried out a number of tests on children who had been vaccinated with this vaccine as well as on those receiving other vaccines and on nonvaccinated controls As shown in Figure 238 about 33 to 38 per cent of the Cutter inoculated vaccines possessed complement fixation antibodies at the time of their bleeding (about a month after a *single* dose of vaccine) probably the result of a subclinical infection In contrast the controls in the same area had only about 5 per cent positives and those who received one dose of Lilly or Parke Davis vaccine were not much higher than the control values

The data from the children in Denver Colorado, who were vaccinated with Cutter material are noteworthy Children receiving 1 0 ml of vaccine (intramuscularly) had the same fre

quency of response as those inoculated with Cutter vaccine in other parts of the country However children receiving one tenth of this dose (intradermally) had a small percentage of positive reactions The explanation may lie in the fa t that this is an example of titrating live virus in children close to the end point of infectivity Thus with 1 ml the frequency of response was 33 per cent and with 0 1 ml 8 per cent On the other hand the route of inoculation may play a role for small amounts of virus intro luced intradermally may produce less trauma and the virus may not multiply as readily as if the inoculation were intramuscular

The results in 1955 shown in Figure 238 were obtained with one dose of vaccine and from our observation at least the vaccines other than Cutter were not of unusually high potency Other observations that we have made with va cine of unusually high potency are illustra tive of the kind of response that one may get with a killed vaccine Figure ?39 shows that children receiving Va cine P (3 doses on days 0 7 and 4?) had respon led by the 4?th day with respectable levels of neutralizing antibodies in virtually all the children The group studied consisted of 5/ young children whose average age was slightly under 3 years so that the neu tralizing antibody responses particularly those in the 25 triply negative children are impressive.

The complement fixation responses in this group are also shown in Figure 239 where the shaded columns pertain to the 3? children who had some neutralizing antibodies in their pre vaccination specimens Only 1 child among the 32 had complement fixation antibodies before va cination Following 2 doses of vaccine anti bodies appeared in 10 to 22 per cent of the chil dren who had prior neutralizing antibodies but not one of the triply negative children re sponded at this time Following the booster dose on the 42nd day complement fixation anti bodies appeared in as many as 25 per cent of these triply negative children suggesting that the previous inoculations had sensitized them to the antigen

Similar data for 78 children inoculated with another potent vaccine (Q) are shown in Figure 240 Again the first children to respond with complement fixation antibodies come from those possessing neutralizing antibodies prior to vac cination

These data are similar to those which we had observed 3 years ago when we studi d the re sponse of monkeys to formalinized vaccine Even though the complement fixation response after vac ination seems to be transitory its presence must make one extremely cautious in interpret ing the results of a single serum specimen If only single specimens are available it is obvious that large numbers of controls collected at the same time must be studied before one can place an interpretation on the laboratory data

BIBLIOGRAPHY

Melnick J L Ann New York Acad Sc 61 1005 1955

Melnick J L McCarroll J R and Horstmann D M Am J Hyg 63 95 1956

Discussion—Precipitin Test

DR GILLIAN CHURCHER

The serologic tests available at present for the diagnosis of poliomyelitis have their limitations as Dr Lennette has emphasized The ideal test would be simple rapid positive at a very early stage of infection and strictly type specific There is some prospect that this ideal may be realized largely by a test based on the direct virus flocculation reaction which we described recently

The reaction was first demonstrated with virus concentrates and the sera of artificially immunized rabbits by the standard Dreyer tube method used for bacterial agglutination This is certainly the method of choice for diagnostic application but the need for economy of re agents has compelled us to evolve a micro method using dark ground illumination for observation of the reaction This in spite of several disadvantages has had to be used for all our subsequent investigations

In extending our experiments to the investi gation of human sera we have continued to use rabbit antisera for the testing and the standardization of antigen batches The reaction with rabbit antisera has always been strictly specific

To gain some understanding of the floccula tion pattern given by human sera we have titrated them by the chessboard technic The results of titrating a pair of sera taken from a Type 1 case from whom a virus was isolated is shown in Table 139 The sera were taken on the 4th and 26th days after the onset of symptoms In this case heterologous reactions occurred to low titer Some of the human sera have shown no heterologous reaction Because of the early appearance of flocculating antibodies found in many of these sera it seemed impor tant to obtain some idea of the flocculation titers of normal sera A number of sera from healthy adults were tested and in only one instance was flocculating antibody present In fa t several of these sera did have neutralizing antibodies to one or more types of polio virus Therefore we provisionally accepted the presence of even small amounts of flocculating antibodies as being indicative of current or recent infection A small survey of sera from cases in which virus isola tions have been made has shown that flocculat ing antibodies appear in a high proportion This is demonstrated in Table 140 which shows the presence of flocculating antibodies in sera from Type 1 and Type 3 infections The right hand column shows those instances where the homol ogous flocculation titer exceeded any heterolo gous reaction

Table 141 shows the correlation between floc culation and complement fixation results With the proviso that the figures are far too small to permit significant statistical analysis it appears that the flocculation test gives a much higher percentage of correct diagnosis than does the

TABLE 139 POLIOMYELITIS FLOCCULATION REACTION CHESSBOARD TITRATIONS OF SERA FROM TYPE 1 PARALYTIC CASE

BRUN ANTIGEN	4TH DAY SERUM 1/4	1/8	1/16	1/32	1/64	1/128	1/256	26TH DAY SERUM 1/4	1/8	1/16	1/32	1/64	1/128	ANTIGEN SALINE CONTROLS
1/2	+++	+++	++	++	−	−	−	+++	+++	++	+	−	−	−
1/4	+++	+++	++	++	+	−	−	+++	+++	++	+	−	−	−
1/8	++	++	+	+	±	−	−	+	+	+	+	−	−	−
1/16	±	+	+	+	+	−	−	−	±	+	+	±	−	−
1/32	−	?tr	±	±	±	?tr	−	−	−	±	±	?tr	−	−
1/64	−	−	−	±	±	?tr	−	−	−	−	±	−	−	−

TABLE 140 POLIOMYELITIS DIAGNOSIS FLOCCULATION TESTS OF HUMAN SERA

Virus Isolated	Flocc vs Antigens 1	2	3	Homologous Titer Highest
Type 1	8/10	3/8	4/10	5/8
Type 3	2/6	2/5	5/6	5/5

N me at = Numb po t e
D m nat = Numb r t sted

TABLE 141 POLIOMYELITIS DIAGNOSIS CORRELATION OF FLOCCULATION AND COMPLEMENT FIXATION RESULTS

Type of Case	Virus Type Isolated	Number of Sera Showing F+ C+	F+ C−	F− C+	F− C−	Totals
Paralytic	Type 1	4	3	0	0	7
Nonparalytic	Type 1	4	1	0	1	6
Paralytic	Type 3	2	3	0	3	8
Paralytic	Nil	0	2	0	0	2
Totals		10	9	0	4	23

complement fixation test In no case was complement fixation positive when flocculation was negative though the reverse was frequently the case

There are innumerable problems still to be solved before adequate correlative serologic surveys can be undertaken Among others these involve preparation standardization stability storage and inactivation of the virus antigens It appears though that the early appearance of flocculating antibodies before the appearance of complement fixing—or a significant rise in neutralizing antibodies—may be one of the most valuable features of this test but it should be stated quite clearly that we make no claim that flocculation provides as yet a reliable diagnostic test—merely that it may do so in the near future

Acknowledgments are due to Dr Alastair Dudgeon and Dr Ann Peach of St George s Hospital Medical School London S W 1 who supplied the sera of poliomyelitis cases with the results of complement fixation and neutralization tests

BIBLIOGRAPHY

Smith W Sheffield F W Churcher G and Lee L H Lancet 2 163 1956
Smith W Sheffield F W Lee L H and Churcher G Lancet 1 710 1956

DISCUSSION

Prof Dr Bernkopf I shall confine myself to a short report of our experience in the isolation of polio and other enteric viruses for diagnostic purposes As Dr Lennette has stressed the choice of a suitable cell system is of primary importance We have used human amnion cells extensively and have found them to be highly satisfactory The ready availability of placentas everywhere and the simple methods needed for the preparation of the cultures make the human amnion useful for many purposes There should be no difficulty in having such cultures prepared in any normal hospital laboratory as no special equipment or facilities are required The number of placentas which do not furnish suitable cells is extremely small if a few simple rules as to pH and temperature are adhered to

The susceptibility of amnion cultures for polio and ECHO viruses is high and compares favorably with monkey kidney and HeLa cell cultures A number of Coxsackie B and A 9 strains also do well in them The high susceptibility of these cultures for polio viruses may be seen from the fact that in a series of 102 cases of paralytic poliomyelitis from which only single stool specimens were obtained 88 cases yielded a *cytopathogenic agent 75 of them polio viruses* in which from 13 cases agents were isolated in amnion cultures which were not neutralized by polio antisera some of them apparently mixtures of polio virus with other cytopathogeni agents These mixtures of several viruses represent a special diagnostic problem

The high susceptibilty of amnion cultures for other enteric viruses may be seen from the fact that among 440 stools collected from a normal child population at different seasons of the year no less than 45 per cent of the specimens contained cytopathogenic agents Polio viruses accounted for only 3 5 per cent of them the rest were mostly Coxsackie and ECHO viruses or mixtures of them It is obvious that under these conditions it is extremely difficult to evaluate the diagnostic significance of cytopathogenic nonpolio agents isolated from the stools

We have paid special attention to the possibility of using differences in the cytopathologic effects caused by the various virus types in amnion cultures for a preliminary differential diagnosis If infected cultures are examined with low power stained or unstained in the culture tubes before the later stages of degeneration set in it is frequently possible to arrive at a tentative identification of polio Coxsackie or ECHO virus types by using the morphologic changes only Of course it is understood that final identification always must be based on the neutralization of the cytopathogenic effect by specific antisera With this reservation it may be said that a number of characteristi s differentiate the changes caused by the various virus types

Time does not permit giving a detailed description of these changes *I shall mention only* the fine ramifi ations of cell processes which appear with great frequency in polio-infected cells while they are absent or rare following Coxsackie or ECHO virus infection also a sharp separation of ecto and endoplasma which is characteristic for certain Coxsackie strains and the delayed or unequal changes following ECHO virus infections in which completely normal looking cells persist for extensive periods next to severely damaged elements Completely different characteristic pictures are produced by adenoviruses herpes virus and others It seems to us that these differences in cytopathology produced by various virus types in tissue culture have a potential value in diagnostic work which has not yet been fully exploited

Prof Blaskovic Dr Lennette s paper described the situation prevalent in practice emphasizing all the difficulties in determining the proper diagnosis of patients *suffering* from a virus infection of the central nervous system This holds true mainly in the group of nonparalytic infections simulating poliomyelitis Analyses made by Dr Lennette have shown the necessity of extending the examination on the group of neurotropic viruses which are common in a particular region or country and can simulate poliomyelitis

In Czechoslovakia differential diagnosis of

poliomyelitis is made in relation to tick borne encephalitis mumps lymphocytic choriomeningitis and the Coxsackie group The diagnosis of ECHO infections has not been carried out in central laboratories until now nor has sufficient attention been paid to the primary infection of the central nervous system with herpes simplex virus

As I mentioned attention is paid to the diagnosis of tick borne encephalitis particularly since the transmission of the infection was confirmed epidemiologically by the tick bite and by milk of infected goats and cows as well It was shown experimentally that the virus is excreted by milk when some days previously a virus suspension had been administered subcutaneously or intradermally as is shown in the following tables

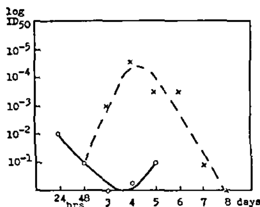


Fig 241 Virus concentration in blood (—) and in milk (- -) of experimentally infected goat

Table 142 Complement Fixation Tests of Patients Suspected of Infection with Tick Borne Encephalitis (Central Moravia)*

Year	No of Tested Samples	Persons	No of Samples Positive	Not Suitable
1953	569	445	85	9
1954	756	393	138	14
1955	733	505	76	17
Total	2 058	1 343	299	40 (1 44%)

J ! ‹ck L a !F ck L Ztsch Imm n tat f sch 113 761 1956

Table 143 Isolation of Tick Borne Encephalitis Virus (Czechoslovak Strain) from Blood Milk Urine and Stool of Experimentally Infected Goats

No of Animal	Material	Interval After Infection 6 Hrs	24 Hrs	48 Hrs	3 d	4 d	5 d	6 d	7 d	8 d
180	Blood	●	●	●	●	●	●	–	–	–
	Milk	–	–	●	●	●	●	●	–	–
	Urine	–	–	–	–	–	–	–	–	–
	Stool	–	–	–	–	–	–	–	–	–
876	Blood	●	●	●	●	–	–	–	–	–
	Milk	–	–	–	●	●	●	●	–	–
875	Blood	–	●	●	●	●	–	–	–	●
	Milk	–	–	●	●	●	●	●	●	–
184	Blood	–	●	●	●	●	–	–	–	–
	Milk	–	–	–	–	–	●	●	●	–

● = Virus isolated
— = Not isolated

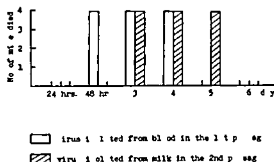


Fig 242 Isolation of tick borne encephalitis (Czechoslovak strain) virus from blood and milk of experimentally infected cow

Table 142 shows surveys of samples collected in a laboratory of central Czechoslovakia in 1953 1954 and 1955 You see the positive results of samples in each year Now let us consider virus distribution of tick borne encephalitis in the blood and the milk of experimentally infected animals

Table 143 shows the viremia after the infection of goats Virus was found in the blood until the fifth day and was excreted in the milk from 48 hours to 8 days Figure 241 shows the virus present in milk and blood of the goat when estimated in titer As is seen it reaches titers which are quite high

The same experiments were run in sheep and you can see (Table 144) a similar picture

If the cow is infected (Figure 242 shows the first experiment) the virus could be detected not only from blood but also in the milk for a couple of days

We are paying attention to this infection because it is sometimes rather difficult to differentiate clinically the polio and the tick borne encephalitis infection in sporadic cases The seasonal occurrence of tick borne encephalitis lasts from April to October and reaches its peak in July and August when polio cases occur

I shall not speak of the diagnosis of polio by virus recovery and complement fixation tests. It is done by methods which are used elsewhere I shall mention only that in the Prague laboratory more than 100 polio viruses were recovered from stool specimens and the same holds true for the differential diagnosis of lymphocytic choriomeningitis which has been established in our country since 1949 Of Coxsackie viruses, 13 strains of different types were recovered in our laboratory last year

BIBLIOGRAPHY

Gresikova M Excretion of tick borne encephalitis virus by goat milk Vet Cas 6 177 1957
——— Excretion of tick borne encephalitis virus by milk of sheep Cs mikrol 2 386 1957
——— Isolation of tick borne encephalitis virus from blood and milk of subcutaneously infected cow Cs mikrol 2 387 1957
Smorodincev A A *et al* Epidemiologiceskie osobennosti dvuchvolnovogo virusnogo meningoencefalita ZMEI 5 54 1953
van Tongeren H A E Excretion of virus by milk of the experimentally infected goat Arch ges Virusforsch 6 158 1955

Table 144 Isolation of Tick Borne Encephalitis Virus (Czechoslovak Strain) from Blood and Milk of Experimentally Infected Sheep

Sheep No	Material	Interval After Infection 24 Hrs	48 Hrs	3 D	4 D	5 D	5 D	7 D	8 D	9 D
867	Blood	—	●	●	—	—	—	—	—	—
	Milk	—	●	●	⊕	⊕	—	—	—	—
868	Blood	●	●	●	●	—	—	—	—	—
	Milk									
869	Blood	●	●	●	●	●	—	—	—	—
	Milk	—	●	⊕	●	●	●	⊕	—	—

● = Virus isolated in the 1st passage
⊕ = Virus isolated in the 2nd passage
— = No virus isolated

Dr Gard Like Dr Salk I would like to comment on the diagnosis of immunity rather than the diagnosis of infection

Inactivation of virus by antibodies is a much slower process than usually is realized The reaction rate is highly temperature-dependent and is not the same in all virus antibody systems

In a mixture of polio virus and human or guinea pig immune serum the full effect is not attained until after about 6 hours at 37 C at refrigerator temperature after about a week In the conventional neutralization test where the virus serum mixture is kept at room temperature for 30 minutes or an hour the reaction has hardly even begun What is measured by this test is mainly the effect of antibody carried over into the test medium—experimental animals or tissue cultures—and at the level of the final dilution in the respective medium Therefore the conventional test is not suited for a demonstration of low levels of serologic immunity

An average thirtyfold increase in sensitivity can be achieved if the reaction between virus and antibody is allowed to proceed to completion before the reaction mixture is seeded into the test medium This technic (immuno-inactivation) as distinct from the conventional test will be described in detail in a forthcoming paper

Immuno inactivation may not be of particular importance in routine diagnostic work However it fills a purpose in the search for traces of antibody as an indicator of previous exposure to the virus Thus in the evaluation of effectiveness of vaccines it is of great importance that the existence of preinoculation immunity can be excluded which according to our experience is by no means possible with the aid of the conventional test So far we have been unable to find a case in which so-called hyperreactivity was not associated with the presence of antibodies demonstrable by a sufficiently sensitive technic In particular appearance of heterologous neutralizing antibodies in the course of natural infection or during monotypic artificial immunization was observed only when a preexisting low level heterologous immunity was demonstrable I would like to add also that the concept of valency of antibodies or avidity of antibodies ought to be considered after studies in which proper attention was paid to the time relationships of the neutralization action

Dr Gear One of the objects of the Polio Research Foundation of Southern Africa was to provide a service for the diagnosis of poliomyelitis This service now provides for the Union of South Africa the territories of southern Africa on occasion for countries farther afield and also the South Sea Islands From 1945 to 1957 monkeys were used for these tests They are freely available in South Africa but even so the number of tests which could be undertaken was limited Since 1952 the tissue-culture technic has been applied to this purpose and with its introduction a considerable expansion in the scope of study was possible It has been our aim to establish the diagnosis of every notified case of poliomyelitis In particular we have studied the cases admitted to the Johannesburg Fever Hospital with a diagnosis of poliomyelitis and have worked in close collaboration with Dr Jackson the senior physician Our laboratory staff concerned know personally every case which they study Such collaboration between the physician and the laboratory worker as Dr Lennette has emphasized is essential for best results

As our knowledge has increased expansion of the facilities has progressively increased too and the following teams have now been established to meet the needs the polio virus team the Coxsackie virus team and the ECHO virus team These teams of course are necessary because the diseases caused by these groups of viruses the Coxsackie virus and the ECHO virus may simulate paralytic poliomyelitis on occasion and more often nonparalytic poliomyelitis Then there is an arthropod borne virus team because many of the arthropod borne viruses may simultaneously simulate poliomyelitis So may animal diseases transmissible to man because on occasion rabies may simulate bulbar poliomyelitis Then there is the serology department which is responsible for the neutralization tests the complement fixation test and perhaps in the future the precipitin test

In addition to these virus teams the facilities of bacteriologic hematologic pathologic and histology departments have also proved to be necessary Such an approach demands a large organization and only a large institution with

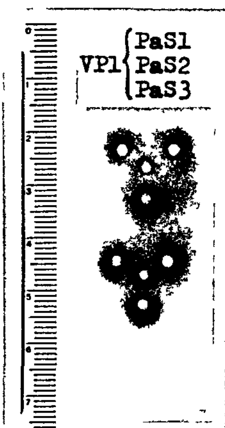
TABLE 145 POLIO VIRUS ISOLATIONS AND TYPING OF STRAINS FROM THE UNION OF SOUTH AFRICA 1955 57

YEAR	1	2	3	TOTAL
	POLIO VIRUS TYPES			
1955	77	5	48	130
1956	655	25	50	730
1957 (Jan Apr)	376	5	16	39
Totals	1 108	35	114	1,25

all these facilities can undertake such a comprehensive service

The results obtained have been of some interest Apparently the service is not yet comprehensive enough because in the investigation of an outbreak of fever which occurred at Durban in spite of a detailed study of many cases the etiology was not found I wonder whether or not it would be necessary to set up a department of psychological medicine.

In the recent epidemics of poliomyelitis over 1 000 virus strains were isolated I will not give the results in detail (see Table 145) However it is of some interest to note that our isolation rate appears to be much higher than that reported by Dr Lennette In particular mention might be made of the studies carried out in cases occurring in the city of Victoria investigated in collaboration with Dr Nelson Of 83 cases studied there virus was isolated 100 per



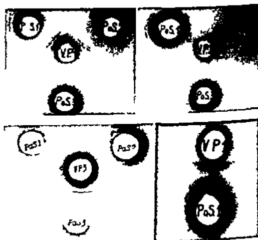


FIG 243 (*Left*) Microscopic slide showing how holes of 2 mm in diameter are punched out in a solidified layer of 1 per cent agar solution (*Top center*) Two precipitation lines obtained with polio virus Type 1 (Strain 6 186) when reference monotypic sera are used (*Top right*) Two precipitation lines obtained with polio virus Type 2 (Strain MEF_1) when reference monotypic sera are used (*Bottom center*) Single precipitation line obtained with polio virus Type 3 (Saukett strain) when reference monotypic sera are used (*Bottom right*) Difference in intensity when 2 precipitation lines are observed (as in the case of polio virus Types 1 and 2)

cent Perhaps the higher isolation rate can be related to the more virulent more cytopathogenic strain of virus responsible for the recent epidemic

Also it is of interest to note that in an outbreak in which one case occurred in a nursery school 70 per cent of the other children were found to be infected It may be of some interest to report that in 1954 Coxsackie Group B Type 4 virus was isolated from the cerebrospinal fluid of a number of cases diagnosed as nonparalytic poliomyelitis

The following summer (in the Northern Hemisphere) the same virus was isolated in Holland In 1955 we isolated from the cerebrospinal fluid of cases a virus which we called a new type Coxsackie A virus Subsequently this virus was shown to be identical with the ECHO Type 9 virus responsible for the extensive epidemic in Western Europe and North America It seems that it would be worthwhile to trace the spread of infection from one area to another from one country to another and possibly from one hemisphere to another It seems worthwhile that international arrangements should be made in the same way as they have been made in the case of influenza and poliomyelitis to enable this to be done Perhaps the International Poliomyelitis Congress may undertake that responsibility

Prof Grasset For the study of the antigenic constitution of the different types of polio viruses and the research of precipitating antibodies in sera the author used the Ouchterlony method of immunoprecipitation Because the usual technic with Petri dishes requires too large quantities of viral antigen for routine use we have elaborated a microtechnic of double diffusion in agar gel which necessitates only very small amounts of material Holes of 2 mm in diameter are punched out 3 to 4 mm apart in a thin layer of a 1 per cent agar solution (solidified on the surface of a routine microscopic slide (Fig 243 *left*) Into separate holes are introduced respectively 0 005 ml of serum under test and 0 005 ml of a high titer polio virus suspension (obtained from culture of infected K B cells) concentrated 100 times by ultracentrifugation The double diffusion results in the formation of precipitation lines in the agar when the serum contains antibodies specific to

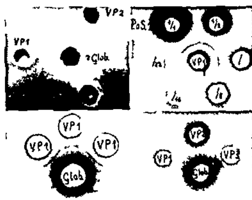


Fig 244 (*Top left*) Precipitation of the 3 types of polio virus by gamma globulins (*Top right*) Slide illustrating how it is possible to evaluate the precipitation rate of the serum by introducing serum in decreasing concentrations into holes surrounding the central hole containing the viral antigen (*Bottom left*) Continuous precipitation line when the same antigen is disposed in the 3 adjacent holes (*Bottom right*) Precipitation lines crossing each other when 3 different types of antigen are examined

the polio virus type introduced into the reaction The lines appear after 18 to 24 hours at 20 C The preparations can be dried and stained and thus can be kept as documents

When reference monotypic sera are used in this system 1 or 2 precipitation lines are observed with polio virus Type 1 (strain 6 186) (Fig 243 *top center*) 2 precipitation lines with polio virus Type 2 (strain MEF$_1$) (Fig 243 *top right*) and a single line with polio virus Type 3 (Saukett strain) (Fig 243 *bottom center*) when these are confronted with their respective monotypic serum

When 2 precipitation lines are observed (as in the case of polio virus Types 1 and 2) they differ in intensity (Fig 243 *bottom right*)

From these experiments it appears that the precipitation reactions are type specific and thus can be used either for the typing of poliomyelitis viruses or for the detection of specific precipitating antibodies

Gamma globulins of human origin as well as sera from rabbits guinea pigs and human beings vaccinated with the Salk vaccine precipi

tate in most cases the 3 types of poliomyelitis (Fig 244 *top left*)

Sera from nonimmunized rabbits and guinea pigs give no reaction with polio virus antigens

When serum in decreasing concentrations is introduced into holes surrounding the central hole containing the viral antigen it is possible to evaluate the precipitin rate of the serum (Fig 244 *top right*)

Furthermore the technic permits comparison of 1 or more viral antigens on the same slide

Figure 244 bottom left shows a continuous precipitation line when the same antigen is dis posed in the 3 adjacent holes On the other hand Figure 244 bottom right shows precipi tation lines crossing each other obtained when 3 different types of antigens are examined

The assays made to separate the antigenic con stitutents of diverse types of polio virus by elec trophoresis in agar did not demonstrate the presence of constituents other than those already observed

The precipitin microreaction in agar has the following characteristics and advantages

1 The reactions obtained are specific Pre cipitating antibodies of a serum are brought into evidence the typing and the titer of these antibodies are determined in less than 24 hours

2 The method is simple and can be per formed rapidly The live antigen by its prepa ration can be conserved for many months in refrigeration without any adjuvant and thus is ready for immediate use

3 Minute amounts of material are used (0 005 ml of antigen and of serum) Up to 6 different sera can be tested on 1 slide (6 holes containing the sera are disposed around a sev enth containing the antigen)

4 The preparations dried and stained on the slides can be kept indefinitely for documentary evidence

Studies are being carried out to determine the relationship existing between the precipitating antibodies thus brought into evidence and the antibodies demonstrated by the seroneutraliza tion tests

Dr Johnsson As we heard from Dr Sved myr the other day there is a heterologous anti body response in patients sera as regards polio virus and ECHO virus Type 6 This seems to have a general validity for enteric viruses

Table 146 shows how complement fixation antibodies against ECHO 6 virus using a con centrated living antigen from monkey kidney tissue fluid were found in 92 per cent of patients if homologous virus was isolated from feces If no virus or heterologous virus was recovered antibodies were found also in 92 per cent and in 2 out of 3 patients from whom Coxsackie B-4 was isolated there was a significant rise in complement fixation antibodies against ECHO 6 antigen These observations were an indica tion for further study of this phenomenon Table 147 shows how in the sera of B 3 excretors there was a fourfold or greater rise in comple ment fixation antibody titer against ECHO 6 in 73 per cent

The same kind of heterotypic antibody re sponse was also found between different ECHO types Table 148 lists a series of complement fixation tests on paired sera from virus positive

Table 146 Occurrence of Neutralizing and Complement Fixing Antibody Response to ECHO Virus Type 6

Diagnosis	Type of Agent Isolated	Number of Patients With Antibodies of Total Number Examined: Neutralization	Number of Patients With Antibodies of Total Number Examined: Complement Fixation	Number of Patients With Significant Rise In Titer: Neutralization	Number of Patients With Significant Rise In Titer: Complement Fixation
Aseptic meningitis	ECHO 6	36/36	36/38	14/18	7/13
	Coxsackie A 7	0/2	1/2	0/0	0/0
	Coxsackie B-4	3/[illegible]	6/6	0/4	2/3
	No virus isolated	6/14	11/12	4/6	1/4
Others	ECHO 6	2/2	3/4	1/1	0/1
	No virus isolated	5/18	16/17	2/8	0/5

TABLE 147 OCCURRENCE OF COMPLEMENT FIXING ANTIBODY RESPONSE TO ECHO 6 IN PATIENTS FROM WHOM COXSACKIE B 3 WAS ISOLATED

CASE NO	ONSET	DATE OF SERUM	CF TITER AGAINST ECHO 6	NEUTRALIZATION TITER† AGAINST COXSACKIE B-3	DATE OF SERUM	CF TITER AGAINST ECHO 6	NEUTRALIZATION TITER AGAINST COXSACKIE B 3
CV 1073	11/3/1950	9/15	4	1 8	11/29	64	2 4
CVI 933	8/20/1952	8/19	4	<1 0	9/1	2	2 5
CVII 40	8/25/1952	8/27	2	<1 0	9/13	16	2 5
CVII 41	8/25/1952	8/27	≦ 2	<1 0	9/13	16	1 5
CVII 42	8/25/1952	8/27	16	1 0	9/16	16	2 5
CVII 68*	8/25/1952	8/31	16	1 7	9/13	64	2 1
CVII 82	8/30/1952	9/1	≦ 2	<1 0	9/13	16	2 0
I 246	9/2/1952	9/5	≦ 2	<1 0	9/9	8	2 3
J 13	9/15/1952	9/17	32	1 0	10/1	128	1 7
II 189	9/24/1952	9/24	16	2 7	10/15	32	1 9
II 247	9/28/1952	10/4	8	1 5	10/16	32	2 3

N gat v n h m n mb y n c l g ulture
Neg t l g c l ul t d d g to R d d M n h

TABLE 148 THE OCCURRENCE OF FOURFOLD RISES OR GREATER IN COMPLEMENT FIXING ANTIBODY RESPONSE IN VIRUS POSITIVE PATIENTS AGAINST 6 DIFFERENT VIRUSES

VIRUS ISOLATED	ANTIGENS USED IN COMPLEMENT FIXATION TESTS				
	ECHO 4	ECHO 6	ECHO 9	COXSACKIE B 3	COXSACKIE B-
ECHO 4	8/11	5/11			7/11
ECHO 6		10/20	1/12	4/14	3/14
ECHO 9		6/15	8/14	6/14	3/14
Coxsackie B 3		9/13	0/12	3/12	/12
Coxsackie B 4	5/10	6/11	6/11	4/11	6/11
Coxsackie A 7	1/1	2/8	0/8	0/1	1/8

TABLE 149 THE OCCURRENCE OF FOURFOLD RISES OR GREATER IN COMPLEMENT FIXING ANTIBODY RESPONSE AGAINST 1 UP TO 5 DIFFERENT ANTIGENS

VIRUS ISOLATED	1 ANTIGEN HOMOLOGOUS	1 ANTIGEN HETEROLOGOUS	2 ANTIGENS	3 ANTIGENS	4 ANTIGENS	5 ANTIGENS
ECHO 4	2	3	2	3		
ECHO 6	3	2	3	0	1	
ECHO 9	4	3	1	3	2	
Coxsackie B 3	0	6	4	0	0	
Coxsackie B 4	0	2	4	1	2	2
Coxsackie A 7		1	0	1		

patients and Table 149 indicates the occurrence of fourfold or greater rises in complement fixing antibody response against from 1 to 5 different antigens Notice that in sera from B 3 excretors where only 1 antigen gave a significant antibody response this in all cases was heterotypic In 2 B 3 excretors there was a significant rise against 5 antigens These 2 cases also have a significant rise against all 3 polio virus types

I leave the question open as to whether this phenomenon depends on the occurrence of a common antigen for enteric viruses or on an amnestic reaction or on some other factors In any case simultaneous infections can be excluded

Obviously complement fixation tests with antigens of this kind are useless for diagnostic purposes as regards enteric viruses and we must rely on isolation of virus

Dr Likar I would like to give a short account of results obtained difficulties encountered and mistakes made in a routine virus laboratory covering an area of 1 5 million inhabitants in the northern part of Yugoslavia called Slovenia The results refer to 157 patients in whom the final clinical diagnosis was paralytic poliomyelitis and to 750 cases of aseptic meningitis All the cases were reported between January 1 1955 and January 1 1957 Emphasis will be laid on a comparison of the etiologic agents in the various neurotropic virus infections in 1955 and 1956 The first of these was a nonepidemic year while the second was that during which the greatest poliomyelitis epidemic yet recorded occurred

Initially serologic methods and isolation technics for the detection of infections with LCM herpes simplex mumps and Russian spring summer meningo-encephalitis viruses were used At the beginning of this survey isolation procedures for poliomyelitis viruses and some other cytopathic agents were added and performed routinely

Fibroblasts grown from minced human embryonic skin and muscles and in a few experiments trypsinized human amniotic membranes were used for the isolation of cytopathic agents In many instances material was inoculated both in suckling mice and tissue culture but as the source of suckling mice was limited we attempted the virus isolation on suckling mice only on request of clinicians

Some experiments were made to test our technic of virus isolations with the optimal material for the primary isolations obtained in 1956 from brain of fatal cases The influence of repeated attempts of virus isolations and subpassages the size of inoculum and that of cortisone were investigated

From 31 specimens 6 were found to be positive for polio virus at the first attempt for virus isolation Repeated subpassages of negative specimens did not prove to be very helpful but repeated first inoculations of material were found valuable for 5 strains were isolated on the second attempt for isolation from the specimens already tested and found negative for virus In 2 cases larger inocula—1 0 ml instead of 0 1 ml—gave evident cytopathic changes 2 to 3 days earlier but no new strains were isolated using larger inocula Addition of cortisone did not show any visible influence on the virus or tissue cultures in doses up to 0 25 mg per ml of the growth medium

In Table 150 the final results of polio virus isolations from brain of fatal cases are given In a great majority the strains were isolated when the patient died with the diagnosis paralytic poliomyelitis A small number of cases of sudden death were investigated for the presence of polio viruses as they occurred at the time of the outbreak of poliomyelitis and were suspected by clinicians to be poliomyelitis In one case when the patient died with the diagnosis poliomyelitis it was subsequently established that the death was accidental and caused by an electric shock

Table 150 Polio Virus Isolated From Brain of Fatal Cases in 1956

Paralytic Poliomyelitis		Encephalitis		Sudden Death	
No Pos	No Neg	No Pos	No Neg	No Pos	No Neg
10	4	1	5	0	8

TABLE 151 VIRUSES ISOLATED IN 1955

Clinical Diagnosis	Material	No of Specimens Tested	Polio	RSSE	Unidentified
Paralytic poliomyelitis	Feces	36	22	0	0
Aseptic meningitis	Feces	14	10	0	2
	Blood	22	0	5	1
Encephalitis	Brain	17	1	8	0
	Blood	11	0	1	1

TABLE 152 VIRUSES ISOLATED IN 1956

Clinical Diagnosis	Material	No of Specimens Tested	Polio	RSSE	Unidentified
Paralytic poliomyelitis	Feces	409	62	0	1
	Brain	15	10	1	0
Aseptic meningitis	Feces	457	24	0	1
	Blood	58	0	2	7
Encephalitis	Brain	8	1	1	1
	Blood	7	0	3	1

TABLE 153 POLIO VIRUS TYPES ISOLATED FROM PATIENTS IN 1955 AND 1956

Clinical Diagnosis	Year	No of Specimens Tested	Poliomyelitis Virus Types 1	2	3
Paralytic poliomyelitis	1955	36	17	0	5
	1956	424	67	1	4
Aseptic meningitis	1955	36	8	0	2
	1956	515	19	1	4
Encephalitis	1955	28	1	0	0
	1956	15	1	0	0

Recently the above isolations were repeated using trypsinized human amniotic membrane the results did not improve

The results of virus isolations are given in Tables 151 and 152 the first showing the numbers of specimens and positive isolates for 1955 when a small number of poliomyelitis cases was recorded and the second for 1956 when an outbreak was observed Table 153 gives the results of typing of the strains isolated in 1955 and 1956

Type 1 polio virus dominated in the epidemic year 1956 as well as in 1955 The percentage of positive isolates of polio viruses is low not surpassing 60 per cent in patients with the diagnosis paralytic poliomyelitis and being just over 10 per cent in patients with the diagnosis aseptic meningitis Some of the reasons for

the low percentage of virus isolations are as follows

1 The specimens were not collected at the best time so that the virus content of the stool specimens varied and in a number of cases was too low to be detected with the present technic Some of the tubes inoculated in positive cases remained negative throughout the incubation period in over 20 per cent of the Type 1 positive tests

2 On many occasions an intermittent excretion of polio virus was observed in 1955 the total isolation rate from feces of paralytic cases amounted to 60 per cent while in 1956 it did not reach 15 per cent but breaking these percentages down considering the individual patient polio virus was isolated in 1955 from 85 per cent of patients with the diagnosis paralytic poliomyelitis and 55 per cent in 1956

3 The diagnosis of poliomyelitis submitted with the specimen to the laboratory had not been supported in every case by the subsequent clinical course and laboratory data Even in cases when epidemiologically and clinically a diagnosis of poliomyelitis was indicated the viruses isolated were not always polio viruses In a case of bulbar paralysis in an ambulance driver who died at the peak of the outbreak an agent was isolated from the brain which was not polio virus and did not belong to the Coxsackie or the ECHO families and was not RSSE mumps herpes simplex or LCM virus

4 Already during 1953 and 1954 a few strains of Russian spring summer meningo encephalitis viruses were isolated and identified In 1955 and 1956 21 strains were isolated which were antigenically similar to if not identical with the viruses of the group of Russian spring summer meningo-encephalitis or louping ill The findings in 1955 differed greatly from those obtained in 1956 In 1955 nearly 50 per cent of specimens tested were found to be positive for RSSE viruses whereas in 1956 less than 9 per cent of specimens tested were positive for virus. The difference is probably due to the fact that in 1955 the endemic tick borne meningo-encephalitis was prevalent and in 1956 with the poliomyelitis and the ECHO aseptic meningitis outbreak the picture changed and many unsuccessful attempts were made to isolate the virus.

5 Over 10 per cent of all strains isolated and shown in Tables 151 and 152 could not be identified They were cytopathic agents with variable pathogenicity for white mice the majority being nonpathogenic We have reasons to believe that a small number of these strains belonged to the Coxsackie family but at least one half to the ECHO viruses as they were isolated mainly in an outbreak of aseptic meningitis which was later proved to be due to ECHO virus Type 9

The complement fixation test for poliomyelitis was not used in our laboratory and the detection of neutralizing antibodies was in the present state of the clinical diagnosis not very helpful Nevertheless complement fixation tests for other viruses were performed routinely and found to be valuable on many occasions The best evidence for this presumption is shown in the results summarized in Table 154

In 10 per cent of serologically proved cases of RSSE and in 10 per cent of serologically proved cases of mumps the original diagnosis was paralytic poliomyelitis These percentages are much higher in respect to the diagnosis aseptic meningitis being 46 per cent for RSSE and 62 per cent for mumps virus

It seems obvious that serologic tests for those viruses known to produce diseases of the central nervous system in the area of the diagnostic

TABLE 154 CORRELATION BETWEEN THE CLINICAL DIAGNOSIS AND THE SEROLOGIC RESULTS

CLINICAL DIAGNOSIS	No OF POSITIVE COMPLEMENT FIXATION TESTS FOR INFLUENZA	RSSE	LCM	MUMPS	PSITTACOSIS
Influenza	166	4	3	1	1
Pneumonia	46	10	6	0	5
Aseptic meningitis	26	133	25	96	1
Encephalitis	0	91	7	23	0
Poliomyelitis	1	29	1	15	0
Status febrilis	15	31	28	19	2

laboratory are a precious aid in raising the percentage of virologically diagnosed neurotropic illnesses

Finally I should like to mention how serologically the etiology of a viral infection of the central nervous system can be elucidated In 1956 we met a form of aseptic meningitis which could not be differentiated clinically from aseptic meningitis caused by the tick-borne RSSE viruses The laboratory was helpless to diagnose the illness for 10 months in spite of the fact that a few strains were isolated from the blood and the cerebrospinal fluid of some patients Paired sera were investigated by a number of laboratories for Coxsackie viruses but no definite increase of antibodies could be demonstrated Through the courtesy of Dr Svedmyr from the Virus Department of the Central Bacteriological Laboratory of Stockholm we obtained a strain of ECHO Type 6 but convalescent sera tested against this virus did not demonstrate neutralizing antibodies Dr Boissard from the Central Public Health Laboratories Colindale kindly sent us a strain of ECHO virus similar to the Type 9 and named Squirell In 14 paired sera still left a definite increase of titer of antibodies for the virus could be demonstrated and later over 700 sera of convalescents were collected and showed the presence of specific neutralizing antibodies in high titers in 95 per cent Even reference laboratories cannot be very helpful on such occasions but international co operation is needed

Dr Meenan I have two major points for consideration First in the future there may be a change in the type of diagnostic work which we will be called on to do Simultaneously with a decline in paralytic poliomyelitis the recognition of the nonparalytic disease and of the symptomless virus excretor will become increasingly important Unless we examine these intensively we face the danger of driving the virus underground for the paralytic case still represents the best index of its activity and it will always be essential that we should know what viruses are circulating and at what level in our communities

The second point is the nature of the specimens which we should use for virus isolation Using fecal specimens in 1956 we obtained the results shown in Table 155 for nonparalytic cases

The reason for the relatively high isolation rate is that this was a selected group insofar as all of these cases were children—many from the same county—and that during 1956 which was an epidemic year almost any illness in a child was regarded as poliomyelitis and treated as such until the contrary was proved

However I feel that if we confine ourselves to fecal specimens alone we may miss a number of virus excretors in the environment of cases at a time when they may be of considerable importance in the spread of infection In support of this I should like to show the results of studies on one family

Family X	Parents and 13 children
10/14	A—ill
10/15	A—clinical poliomyelitis died B—clinical poliomyelitis
10/16	Mouth and throat swabs collected
10/17	C and D—clinical poliomyelitis 8 admitted to hospital for quarantine
10/18	Two more children quarantined One child (0) not living at home not quarantined
10/19	Feces collected
10/20	B died
10/26	E—clinical poliomyelitis

Child	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Swabs	+	−	+	−	−	−	−	−	+	−	−	+	−	−
Feces	NS	+	NS	+	+	−	−	+	−	−	−	−	+	NS

+ = Virus isolated
NS = No specimen collected

In 3 of the 4 cases who had virus present in the throat it was detected also in the saliva

Table 155 Results for Nonparalytic Cases

Cases	1	2	3	Untyped	Per cent of Isolation
39	[illegible]	2	1	4	74

In specimens collected at only 2 points in time poliomyelitis was therefore isolated from 9 members of this family of whom 1 was apparently not at risk and 1 had died before the investigation began Had feces only and not mouth and throat swabs been examined 2 symptomless members of this family might have been regarded as being free from infection at a time when in fact they might well have been at their most dangerous

As a result of this and similar although perhaps less spectacular studies I feel strongly that more intensive examination of oropharyngeal specimens would be rewarding in diagnosis and could lead to a reorientation of our thinking on the epidemiology of poliomyelitis—which may have been led astray by the sheer mass of virus in the stools

Prof Przesmycki The following are some remarks regarding Dr Lennette s report

1 The percentage of paralytic cases from which the virus could be isolated is somewhat higher in the experience of the State Institute of Hygiene Warsaw than in Dr Lennette s One should stress the point that 61 per cent of samples have been collected prior to the 10th day of illness and in addition the most material has been provided by one of the Warsaw hospitals The positive results amounted in paralytic cases to 77 2 per cent and in the nonparalytic cases to 29 9 per cent 8 (16 6 %) strains of polio and 2 orphan strains were isolated from cases with uncertain diagnosis (see Table 156) The material collected was preserved by refrigeration for 2 hours This table shows how important virologic investigation is for proper diagnosis

2 In some of the countries in addition to poliomyelitis some other neural infections chiefly of an encephalitic character are seen Therefore the number of antigens should be enlarged by addition of the tick borne encephalitis

3 During the study of neuro-infections quite a number of strains of Coxsackie ECHO tick borne encephalitis and other identified strains are isolated As yet the etiologic role of these strains is not sufficiently clear The laboratories meet with great difficulties in the identification of the isolated strains Standard sera are also lacking

In order to meet all these needs I move that a center for the study of neuro infections should be established This institution should work on lines similar to those of the Influenza Center

Also it is of paramount importance that a course for the training of the virologic laboratory s staff prepared for the study of neuro-infections should be organized under the auspices of the WHO

Dr Smadel I have no data to present but I would like to discuss with you the few ideas on where we have been in the diagnosis of polio and some of the other enteric agents and diseases and where we need to go now

Anyone who attended the meeting in Copenhagen may recall that in presenting a general review of the diagnostic procedures in much the same manner that Dr Lennette presented it this morning I was rather hard put to find enough to talk about in the diagnosis of polio and spent a fair amount of time discussing diagnostic procedures for other related CNS diseases

I think all of us should be very happy and grateful to the virologist for his having done so well in a few years in developing a series of very precise research tools such as we have heard

Table 156 Outcome of Tests for Viruses

	Examined	Polio Virus Isolated	Per cent	Orphan Strains Isolated	Period During Which the Material was Isolated
Paralytic cases	127	98	77 2	4	1–10 = 61 3%
Nonparalytic cases	61	18	29 9	2	11–20 = 31 8%
Cases with uncertain diagnosis	48	8	16 6	2	21–30 = 6 9%
Total	236	124	53 4	8	

discussed today I would emphasize though that these are still research tools and that Dr Lennette in presenting his work rightly emphasized the differ nce between the virus diagnostic laboratory and the virus research laboratory pointing out some of the difficulties which the virus diagnostic laboratory faces It would be fruitless to quibble about whether the isolation rate is 60 per cent 70 per cent or 80 per cent We are really not discussing the value of a particular technic We are discussing the value of the entire procedure from the time th phy sician sees the patient on through until the material reaches the sensitive cell Let us merely be grateful that we have such excellent tools and let us continue to use them

Let us also ask about the physician who is not close to the very expensive virus diagnostic labo ratory which also carries with it research or is not fortunate enough to have a friend who is a researcher in poliomyelitis and is willing to do some of his diagnostic work for him What about the man who is a long way from these places? He needs some fairly simple tools They should be reasonably accurate—80 per cent one would settle for The old Weil Felix test is a very valuable thing in the rickettsial diseases it is only about 80 per cent accurate but it is useful This is the sort of thing we need

I wonder whether we do not have enough of the technical know how to begin to get such a test using stool specimens which are about as rich in virus as most of the things we were able to g t until a few years ago and using some of the coated particle technics that we have used very extensively in all sorts of tests in the last few years I do not know whether or not one would be successful in coating the particles with antibody or in coating them with some of the virus in the stools but I think it is worth a try One might get a simple quick test by such an approach

The other thing that we need is a tool to help the epidemiologist—something as good as the typing tool in typhoid fever There is an indication that such a tool might be at hand also M Bride of the National Institutes of Health in Bethesda reported a te hnic a few months ago to the American Society of Ba teri ologists in which he employed the old phage neutralization technic using the plaques of polio mixing virus and antisera and diluting these at different time intervals and getting the reduc tion in plaques with time This suggests at least the preliminary data suggest that this is a simpler way of telling the difference between strains Here is another kind of tool that we need Neither of these tools is ready for use but I think that perhaps before the next Con gress we might have them

Dr Svedmyr Today s topic has already been very well covered still I would like to make two minor comments

1 I would like to ask Dr Lennette as well as Dr Verlinde whether or not the age distri bution among their paralytic patients who were virus positive differed from that of the virus negative ones During the severe epidemic of poliomyelitis in Stockholm in 1953 in 91 per cent of the cases we recovered Type 1 virus from single stool specimens of paralytic children who were below 16 years of age provided that the specimens were procured within 10 days after onset of the disease The corresponding frequency for adults was only 68 per cent A similar age difference was found for the non paralytic cases of aseptic meningitis thus 83 per cent of the children but only 49 per cent of the adults were virus positive—again provided that the stools were taken within 10 days after onset All recovery rates were somewhat lower during the next 10-day period

It seems probable that the children of 10 at least during our epidemic excreted virus in somewhat larger amounts and probably also for a longer time than did the adults I would hesitate to speculate about the mechanism of this phenomenon Possibly it may be correlated to previous experience of polio virus

2 I agree with Dr Johnsson that for the time being it might be wise not to base an etiologic diagnosis of infection with polio virus or for that matter other enteric viruses on the results of a complement fixation reaction alone How ever maybe we should await further clarifica tion of the nature and the frequency of the heterologous reaction which apparently has been encountered in several laboratories before we completely reject the complement fixation test for diagnostic purposes

PROF DR VERLINDE As I pointed out in the discussion on enteric viruses last Tuesday an epidemic of viral meningitis due to ECHO 9 virus occurred simultaneously in the Netherlands with an epidemic of poliomyelitis The stool specimens from all over our country of 11 000 000 inhabitants collected by a large number of local physicians without any control of our diagnostic service were sent to our laboratory and examined virologically Special containers and dry ice were not available for local physicians so that specimens were sent in a variety of bottles by ordinary mail however most of them were sent by special delivery so that the vast majority reached the laboratory within 24 hours

The specimens were routinely examined by inoculation of monkey kidney cultures and suckling mice

Because of the simultaneous occurrence of meningitis due to ECHO 9 virus specimens from patients with meningeal involvement were particularly important for the differentiation between meningitic forms of poliomyelitis and viral meningitis of other etiology

The total number of virus strains isolated was over 1 500 originating from 1 472 patients which indicates that a virus was recovered from most of them on only one occasion As a matter of fact more than one stool specimen from the same patient was received only occasionally

Of these strains over 800 were of the poliomyelitis group and the type distribution was as follows Type 1 91 per cent Type 2 8 per cent Type 3 1 per cent

Since most of the clinical data which accompanied the specimens were insufficient we are trying in follow up to complete them So far complete clinical data of 600 patients are now available and on this basis I would compare Dr Lennette s basis with ours

1 The interval between the indicated onset of illness and the collection of specimens ranged from 0 to 30 days the median interval being 9 days

2 In 66 per cent of 200 patients with paralytic poliomyelitis a polio virus was recovered The viral recovery rate in the first week of illness was over 80 per cent and then in the following weeks decreased gradually Of 400 patients with the clinical diagnosis of nonparalytic poliomyelitis—meningeal involvement and abortive cases—only 10 per cent yielded polio virus and from 46 per cent ECHO 9 virus was recovered

3 In a small number of patients a comparison of the viral recovery rate from stools rectal swabs pharyngeal swabs and cerebrospinal fluid could be made In those who yielded virus from one or more of these specimens the following percentages were found to be positive

When polio virus was recovered the stools were found to be positive in 75 per cent and the rectal swabs from the same patients in 70 per cent The pharyngeal swabs gave 21 per cent, and cerebrospinal fluids were always negative

On the contrary when ECHO virus was isolated we found the stools to be positive in 100 per cent the rectal swabs in only 58 per cent the pharyngeal swabs in 83 per cent and the cerebrospinal fluid in 28 per cent

From this small number of patients it appears that the viral recovery rates from rectal swabs and stools do not differ significantly as far as polio virus is concerned As to the recovery of ECHO virus the viral recovery rate from rectal swabs was inferior to that from stool specimens and even from pharyngeal swabs

DR VOROSHILOVA Laboratory diagnosis in poliomyelitis is being used more and more widely in the Soviet Union In the past 2 years in a number of towns special diagnostic laboratories which work under the general methodical guidance of the Poliomyelitis Research Institute were organized Centralized supply of type specific immune sera and standard strains of virus is provided for these laboratories

Isolation of 1 140 strains of virus has been described 4 of these strains have been classified as a strain of Type 4 Let me discuss this question of Type 4 virus The question of the existence of Type 4 poliomyelitis virus in the USSR arose in 1952 when during an epidemic of poliomyelitis in Karaganda 3 strains of peculiar virus were isolated from stools of patients with paralytic poliomyelitis 2 of the strains (AB and G7) were isolated by direct inoculation of monkeys The third strain (MK) was originally isolated in cotton rats and new born white mice and later adapted to monkeys

Figure 245 presents a diagram of AB strain

СХЕМА ВЫДЕЛЕНИЯ ШТАММА АБ IV ТИПА ВИРУСА ПОЛИОМИЭЛИТА
ИЗ КАЛА БОЛЬНЫХ АХУНДОВА И БЕЛИК

Fig 245 Diagram of isolation of Type 4 AB strain of polio virus

isolation from stools of patients Achundova and Belik who later died from the bulbar spinal form of poliomyelitis A Macacus hybrid Lastic inoculated intraperitoneally and intranasally became ill on the 5th postinoculation day There was a rise in temperature (Fig 246) followed by flaccid paralysis of the lower extremities This monkey's brain was inoculated intracerebrally with positive result to monkey Eder and suckling cotton rats A total of 4 successful passages in monkeys have been carried out Beginning with the second monkey passage their brain and spinal cord emulsions were used concurrently to infect adult and newborn cotton rats and white mice Lines of continuous passages were carried out in suckling cotton rats which proved to be most susceptible to infection with this strain Typical experimental poliomyelitis (Fig 247) was produced in monkeys inoculated with brain suspension from the 10th and 24th passage in suckling cotton rats

C7 strain isolated in September 1952 by means of intracerebral inoculation of monkey with feces of 2 patients with paralytic poliomye

КРИВЫЕ ТЕМПЕРАТУРЫ ОБЕЗЬЯН
ЗАРАЖЕННЫХ ШТАММОМ ВИРУСА
ПОЛИОМИЭЛИТА IV ТИПА
ШТАММ АБ

Fig 246 Temperature curves of monkeys inoculated with Type 4 AB strain



Fig 247 Macacus rhesus Angara inoculated with AB strain brain suspension from the 26th suckling cotton rat passage (Quadriplegy paralysis of muscles of the back)

litis was carried out through 4 more passages in monkeys MK strain was isolated from pooled feces of 2 patients with paralytic poliomyelitis by inoculation of adult cotton rats and newborn white mice As further experiments showed suckling cotton rats were found to be most susceptible to infection then came adult cotton rats and suckling white mice Many attempts to inoculate adult white mice always gave negative results

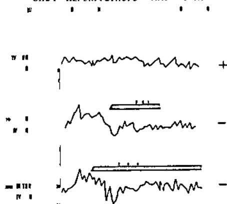
Thus Karaganda strains differed in their pathogenicity from Type 1 and Type 3 strains, as they caused disease not only in monkeys but also in suckling and adult cotton rats and in suckling white mice In contrast to Type 2 strains AB GZ and MK strains are apathogenic for adult white mice Histologic examination of brain and spinal cord from paralyzed monkeys showed lesions typical for experimental poliomyelitis (Fig 248 *left*) Besides lesions in the central nervous system myositis was found in suckling white mice and suckling cotton rats (Fig 248 *right*) A monkey was inoculated with muscle suspension from the 17th suckling cotton rat passage of MK strain Simultaneously another monkey was inoculated with brain from the same suckling cotton rat Both inoculated monkeys came down with severe experimental poliomyelitis

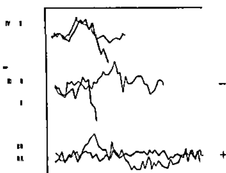
We established the difference of AB and MK strains from Type 1 and Type 3 polio viruses (Figs 249 250 and 251) in cross immunity tests in monkeys and we showed the absence of immunologic relationship between AB and MK strains on the one hand and Type 2 Lansing and OVCH strains on the other (Figs 252 and 253) in experiments on cotton rats

On the basis of these facts and considering the absence of immunologic relationship with



Fig 248 (*Left*) Photomicrograph showing massive degeneration of nerve cells in lumbar anterior horn of macacus rhesus Lastic inoculated with stools of patients Achundova and Belik AB strain (*Right*) Diffuse myositis in a suckling cotton rat inoculated with AB strain



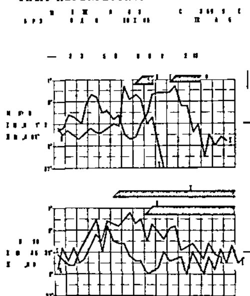


F c ?49 (*Top*) Cross mmun ty test monkeys mmune to Type 1 and Type 3 pol o viru es are infected th AB stra n

F c 250 (*Bottom*) Cross mmun y test monkeys mmune to Type 1 Type 3 and Typ 4 pol o v ruses are infected w th Typ 1 TV stra n

Type 1 2 and 3 stra ns of pol o v ruses a sug gestion was made that these stra ns from Kara ganda should represent the fourth mmunolog c type of pol o v rus

At the same t me some pecul ar t es of the abo e s ra ns were no e l In h stolog c exam nat on of a large number of monkeys in about 50 per cent of cases bes des marked pol o- myelit s les ons n the sp nal cord and the medulla some small inflammatory foc in



F c 251 Cross immun ty tes monkeys mmune to Type 1 Type 3 and Type 4 pol o ruses a e infected v th Type 1 KI F 1 and Type AB nfect on are inocu la ed h Type 3 Leon u

frontal temporal par etal and occ p tal lol es of the cerebrum we e found also there vere mo e extens e les ons n the medulla than ere ob erved n Type 1 pol o rus nfect on AB and MK s ra ns produced myos t s in suck l ng cotton rats and suckl ng vh te m ce exper mental pol omyel t s ha ng been reproduced l y inoculat on of monkeys v th muscles from these paralyzed suckl ngs

In 195(Type 4 AB stra n was sent for study to the Karol nska Inst tute n S ockholm to the Pasteur Inst tute n Par s and to se eral labora tor es in the U S A Accord ng to Dr K Habel and Dr L Looms and also Drs D Horst mann and E Mono l d s our fundamental data on the propert es of AB stra n were confirmed n the r laboratories

In 1956 Doctor Johnsson (Stockholm) found ant gen c s m lar ty bet een AB stra n and Cox sack e A 7 stra n. Th s s m lar ty as confirmed serolog ally n our laboratory too v hen e got all the 24 types of Coxsack e v ruses from Czecho lo ak a

ПЕРЕКРЕСТНЫЙ ИММУНИТЕТ У ХЛОПКОВЫХ КРЫС ИММУНИЗИРОВАННЫХ ШТАММАМИ ВИРУСА ТИПОВ II И IV

[illegible]	[illegible]			
	IV АБ	IV МК	II ОВЧ	II лансинг
IV АБ	>10000	>1000	25	<100
IV МК		>316		0
IV ГЗ		162		12
II ОВЧ	12	14	100000	10000
II лансинг	10	0	100000	10000

ОПЫТЫ НЕЙТРАЛИЗАЦИИ НА ХЛОПКОВЫХ КРЫСАХ ШТАММОВ ТИПА II И IV ИММУННЫМИ АНТИСЫВОРОТКАМИ

Иммунные антисыворотки [illegible]	Тип и штамм вируса			
	IV АБ	IV МК	II ОВЧ	II лансинг
IV АБ	>4		0 67	0
IV МК		>3 4		<1 4
II ОВЧ	0		3 33	3 0
II лансинг	0	<1 4	>3,33	

Fig 252 (*Top*) Cross immunity in cotton rats immunized with Type 2 and Type 4 viruses

Fig 253 (*Bottom*) Cross neutralization test in cotton rats with Type 2 and Type 4 strains



Fig 254 Photomicrograph showing disappearance of the inner group of cells in the lumbar cord of macacus rhesus inoculated with Coxsackie A 7 virus



Fig 255 Coxsackie A 7 virus Paralysis of both hind legs in cotton rat 9th day after infection

Together with Golubeva we inoculated monkeys and adult cotton rats with Coxsackie A 7 virus and the first monkey inoculated with muscle suspension from paralyzed suckling white mice developed fever paresis of lower extremities and typical histologic poliomyelitic changes in the spinal cord (Fig 254) Coxsackie A 7 strain was pathogenic also for adult cotton rats and regularly produced paralyzed animals (Fig 255)

Thus the characteristics of Coxsackie A 7 strain as well as the characteristics of Karaganda AB-4 GZ-4 and MK 4 strains do not correspond to the definition of the Coxsackie group of viruses because of marked pathogenicity for monkeys and cotton rats and capacity to produce poliomyelitislike lesions in the central nervous system of these animals

The capacity to produce myositis in monkeys was established in our Institute not only for strains of Coxsackie A 7 AB 4 but also for one strain of Type 2 polio virus also immunologically distinct from the strain AB-4 The Karaganda strains possess marked neurotropism in monkey experiments consequently one cannot be sure that they will not cause poliomyelitis in human beings Direct isolation in monkeys of virus from patients who died of the bulbar spinal form of poliomyelitis in Karaganda also shows the necessity of new studies directed toward elucidation of the etiologic role of this group of viruses in human disease

Doctor Sven Gard wrote in 1955 The Coxsackie group is apparently not homogeneous it is very probable that some of its present members will be classified differently in the future

We are inclined to think that the data on Coxsackie A 7 strain and AB-4 strain accumulated in the 4 laboratories in different countries

of the world are contrary to the ma n character st cs of the Coxsackie group of viruses and they rather confirm the op n on of the poss b l ty of group ng them nto an independent Type 4 pol omyel t s virus or a spec al v rus of para pol omyel t s infect on Then we have Types 1 and 3 pol o v rus wh ch are str ctly pathogen c for monkeys Type 2 wh ch is pathogen c for monkeys wh te m ce and occas onally may in duce myosit s in suckl ngs then Type 4 Cox sack e A 7 wh ch is pathogen c for monkeys for suckl ng cotton rats and sucklings and then Coxsack e groups of v ruses themselves Further invest gat ons should be carr ed out on the prevalence of v ruses of th s group and also more complete stud es should be made on patho gen city spectrum of o her members of Coxsack e and ECHO groups of v ruses

Basic Problems of Respiratory Distress in Patients with Poliomyelitis

WEDNESDAY MORNING, JULY 10, 1957

(This Session Convened in the Aula of the University of Geneva)

Chairman

DR PIERRE RECHT
European Association
Against Poliomyelitis
Brussels

Speakers

DR J H COMROE JR
University of Pennsylvania
Medical School
Philadelphia

DR. JAMES L WHITTENBERGER
Harvard University
School of Public Health
Boston

DR JERE MEAD
Harvard University
School of Public Health
Boston

DR JAMES F BOSMA
University of Utah
College of Medicine
Salt Lake City

DR CLARENCE W DAIL
Rancho Los Amigos Hospital
Hondo California

Discussants

DR G M ARDRAN
Oxford University
Nuffield Institute for Medical Research
Oxford

DR CLARENCE R COLLIER
Rancho Los Amigos Hospital
Hondo California

DR H HEEMSTRA
University of Groningen
Physiological Institute
Groningen

DR HELMUT M KIRSCHSIEPER
Universitats Kinderklinik
Freiburg

PROF PIERRE MOLLARET
Hospital Claude Bernard
Clinic of Infectious Diseases
Paris

Assessment of Respiratory and Pulmonary Function in Patients with Poliomyelitis

DR J H COMROE, JR

The ideal management of a patient with respiratory failure either acute or chronic requires a physician who has an expert knowledge of respiratory physiology and of pulmonary function

Although the function of the lungs is a simple one—that of pulmonary gas exchange—this function is achieved by a series of processes

Figure 256 top left shows the first of these processes namely that of ventilation and the most important effect here is that there shall be an adequate volume of fresh air entering the respiratory passages the conducting airway down into the small air sacs or alveoli of the lungs where rapid exchange of oxygen and carbon dioxide occurs

Figure 256 top right shows that in addition to the volume of ventilation it is important that there be even distribution of the inspired air so that the air entering the nose and the mouth is distributed equally to all of the millions of alveoli or air sacs in the lung

Figure 256 bottom left indicates the third of these processes namely that of diffusion After air enters the airway and is distributed evenly to the alveoli it must diffuse across the alveolar capillary membrane into the pulmonary capillary blood

Figure 256 bottom right shows the last of these processes Here one has mixed venous blood entering the pulmonary capillaries coming into contact with alveolar gas becoming arterialized and oxygenated very quickly and then leaving as arterial blood It is important that pulmonary blood flow be adequate in volume and also even in its distribution to all the alveoli in the lungs

In addition you see that these processes do require work Work is required on the part of the respiratory muscles to accomplish ventilation Work is required on the part of the right ventricle to push capillary blood through the pulmonary capillaries Therefore pulmonary function is normal only when ventilation diffusion and blood flow are normal and when the work required to accomplish these is also normal These facts are simple these facts are elementary everyone knows them and almost everyone forgets them Therefore I would like to remind you that it is not enough to measure the rate the depth or even the minute volume of respiration and on the basis of this call ventilation normal or abnormal

Figure 257 shows 3 different combinations of alveolar ventilation B is the record of an individual breathing normally 500 cc with each breath 16 times a minute minute volume 8 000 ml A is an individual breathing twice as fast and half as deeply 32 times per minute 250 ml per breath and again he has the same minute volume 8 000 ml C is a subject breathing very slowly and deeply 8 times a minute 1 000 ml per breath again a minute volume of 8 000 ml In each case the minute volume is the same but the useful ventilation that is the alveolar ventilation is different in each In A it is 3,200 ml which is too little in B it is 5 600 which is about right in C it is 6 800 which is too much and results in hyperventilation

Now the reason for these differences in useful or alveolar ventilation is because of the respiratory dead space It is possible to measure the anatomic respiratory dead space

Figure 258 shows one way of measuring the anatomic dead space This is an individual breathing air He takes one deep breath of pure oxygen and at the indicated point breathes out. The air passes through a nitrogen meter which records instantaneously and continuously nitrogen concentration in the expired air The final plateau represents alveolar gas that has been expired later I will mention that this is important in evaluating how evenly air is distributed among the alveoli A represents the washout

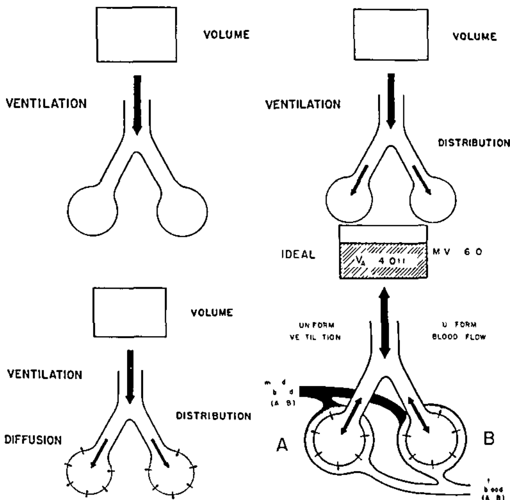


Fig 256 (*Top left*) Ventilation process showing volume of air entering respiratory passages (*Top right*) Even distribution of air entering respiratory passages (*Bottom left*) Diffusion of air into the pulmonary capillary blood (*bottom right*) Changing of venous blood to arterial blood in the pulmonary capillaries

of the dead spa e analysis of this enables one to calculate and measure the anatomic dead space However I would like to point out that even though one knows accurately this anatomic dead space one still does not have all the infor mation that one needs to evaluate ventilation and pulmonary fun tion The reason is that the amount of ventilation that an individual needs mu t be related to his activity at that moment—his oxygen consumption his CO_2 produ tion and this depends on the degree of his muscular activity whether or not he has fever and other factors It is also important to remember that even though the minute volume the dead space and the rate are normal in relation to the amount of activity of the patient there still may be anoxemia there still may be CO retention if the ventilation of the patient is uneven if the pulmonary capillary blood flow is uneven or if the diffusion across the alveolar capillary mem brane is impaired Therefore it is important in some cases to know not only the volume of ven

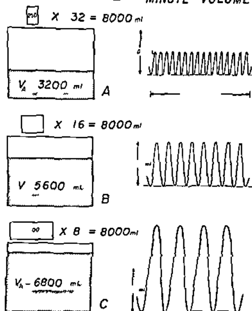


FIG 257 Differ nt combinations of alveolar ventilation

tilation but also whether it is uniform or not and the single breath of oxygen using the nitrogen meter as shown in Figure 258 is a good quick test of the uniformity of the alveolar ventilation

It is also important to think in terms of the diffusing capacity of the lungs It is now possible to measure quickly and easily the diffusing capacity of a patient s lungs This is a test using carbon monoxide according to the old technic of Marie Krogh somewhat modified by Dr Forster in our department

A patient who is breathing air normally expires and then breathes in de ply a mixture of low nontoxic concentration of carbon monoxide and some helium as a tracer gas The individual holds his breath for 10 seconds then blows out The alveolar gas is collected and from this one can make calculations of the diffusing capacity of the lungs One can make these measurements quickly and one can repeat them frequently they involve no blood samples no cardiac catheterization From such data one can have an idea an index of the area of the ventilated alveoli in the lungs that are in contact with pulmonary capillary circulation and one can also have an idea of the thickness of the alveolar capillary membrane

One might ask is it ever necessary to know anything in the face of poliomyelitis excep the volume of ventilation? The answer is yes it is necessary Uneven ventilation may occur owing to inhalation of secretions localized infection in the lung localized edema in the lung or even pneumothorax Impaired diffusion may occur because of pulmonary edema either in the tissues of the lung or the alveoli Impaired diffu

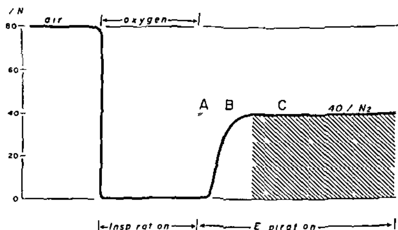


FIG 258 Single breath analysis (nitrogen meter)

sion may occur because of inflammation or pneumonia. Uneven blood flow may occur because of atelectasis, collapse of the lungs or pulmonary embolism or thrombosis.

Are there any simple over-all tests of pulmonary functions that help to evaluate the patient? In order to do this one must know the arterial oxygen and the arterial carbon dioxide. The arterial-oxygen saturation may be measured by an oximeter and is of particular value when one asks the individual to breathe 100 per cent oxygen for about 10 minutes after breathing room air to see how high the arterial-oxygen saturation then rises. It may be possible within a year to measure the oxygen tension or pressure in the arterial blood and to do this much more rapidly than to measure arterial-oxygen saturation. The oxygen electrode has been greatly improved and may come into widespread use fairly soon. The arterial carbon dioxide pressure or tension may be measured without sampling arterial blood if one obtains a sample of alveolar gas after the dead space has been washed out. This CO_2 may be measured either with a infrared analyzer or by very simple chemical technics using the Scholander 5 ml analyzer and an ordinary 5 ml syringe. I believe that in the complete evaluation it is not

Fig 259 Plethysmograph or body box for testing pulmonary function

enough to measure carbon dioxide alone or to measure oxygen alone. If the oxygen alone is measured and is normal it gives no clue as to whether or not the patient is being hyperventilated as he may well be with consequent cerebral vasoconstriction and tetany. If the car

Fig 260 Schematic drawing of plethysmograph

bon dioxide alone is measured and is found to be normal or even low this gives no clue as to an impairment of diffusion because in such cases the CO_2 may be normal or low

There is a new method for testing some aspects of pulmonary function using a body box or a body plethysmograph

Figure 259 shows an individual sitting within the body box a box about as big as a telephone booth The individual sits within the box and breathes air from around him He does not become anoxic because he is there for only a few minutes and there are 600 liters of air in the box The door is then closed and pressures are measured within the box

Figure 260 shows a scheme of the individual within the box breathing through a flowmeter While he is breathing the pressure within the box is measured continuously by a very sensitive electrical manometer (P) and the pressure in the airway is measured by another sensitive manometer (P_1) Flow and pressure are measured simultaneously and recorded on the cathode ray oscilloscope By means of this body box and a number of different technics we have now measured the thoracic gas volume the volume of trapped air within the lungs pulmonary capillary blood flow airway resistance and pulmonary tissue resistance

Figure 261 illustrates the principle involved in one of these measurements that is of measuring airway resistance To measure airway resistance one must know alveolar pressure and flow Flow is measured by a pneumotachograph Alveolar pressure is measured in the body box When the individual breathes in during inspiration the air in the lungs is at a lower pressure then than atmospheric. This air is expanded and this compresses the air in the box so that the gauge shows an increased reading By taking measurements of this during inspiration and expiration one can measure alveolar gas pressure continuously during flow and so measure airway resistance

A large number of pulmonary tests can now be done on patients One can measure the volume of ventilation One can measure its distribution One can measure diffusion One can measure pulmonary capillary blood flow So far as mechanical tests are concerned one can now measure the resistance in the airway the resistance in the pulmonary tissues and the compliance But Dr Mead and Dr Whittenberger who follow me will talk to you more about these measures These new tests of pulmonary function have now been applied to patients who have recovered from poliomyelitis Approximately ¼ of the patients who have had poliomyelitis and respiratory muscle paralysis when they recover developed clinically detectable scoliosis Many surgeons have tried to correct these deformities of the thorax and of the spine. Often they may be very severe deformities. However there has been no systematic attempt to measure pulmonary function in individuals

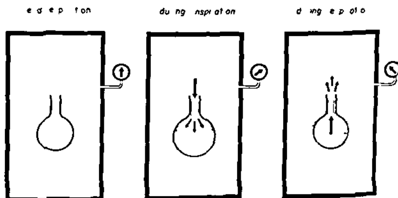


Fig 261 Principle of measuring airway resistance

who have recovered from poliomyelitis and who now have thoracic or spinal deformities Studies are now under way in our department by Dr Thomas Gucker an orthopedic surgeon who has a deep interest in problems of pulmonary physiology This study will take several years in order to follow these individuals as they are treated by long term orthopedic procedures Twenty two patients have been studied so far In a number of these patients the vital capacity is greatly reduced the maximum breathing capacity is greatly reduced the flow rates may be very low indeed The compliance of the lungs is often much reduced The airway resistance measured in the body box is often greatly increased Therefore you see that before any orthopedic procedures are attempted these patients have severe impairment of the function of the lungs and the thorax It is important to find out whether or not the standard orthopedic procedures make these conditions worse In some instances particularly those involved in extensive immobilization by the surgeon pulmonary function has been made much worse during the period of treatment

In the management of poliomyelitis remember that the respiratory difficulties of the patient are not necessarily at an end Even when the patient has recovered from the acute attack and has gone home he may within several years develop scoliosis and severe respiratory impairment on this account Surgeons or other physicians who engage in corrective procedures of the spine and thorax of these patients should consider seriously the pulmonary and respiratory difficulties and make objective tests of pulmonary functions before during and after the orthopedic procedures

Principles of Respiratory Mechanics and Coughing

DR JAMES L WHITTENBERGER

The mechanics of breathing have been little taught in medical schools until the past 5 years This aspect of respiratory physiology is basic to understanding artificial respiration and pressure breathing

In breathing the respiratory muscles must move the thorax and the abdomen the lungs and the gas in the passages within the lungs and the respiratory tract In accomplishing this movement two principal properties of the system are encountered These are *elastic* and *flow resistive* properties of the lungs and the thorax The flow resistive properties include the viscous resistance offered by the pulmonary and the thoracic tissues and the gas flow resistance offered by the air passages

We will consider first the elastic properties exhibited by the lungs and the thorax The elastic recoil of the chest normally tends to expand the lungs and the elastic recoil of the lungs tends to collapse them If the respiratory muscles are entirely relaxed or paralyzed by a relaxing drug or polio these opposing elastic forces are in equilibrium at a level of lung volume which may be called the resting end expiratory volume or the *functional residual capacity* In the upright posture the intrapleural pressure at this volume is approximately 5 cm of water less than atmospheric pressure In the supine position the weight of the abdominal contents pushes the diaphragm in the expiratory direction and the elastic equilibrium between the lungs and the thorax is shifted to a smaller volume Since the lung volume is smaller the elastic recoil is less and the resting end-expiratory intrapleural pressure is closer to atmospheric —perhaps 3 cm of water less than atmospheric

During inspiration the respiratory muscles increase the volume of the thorax and the lungs above the equilibrium volume Thereby the lungs are stretched further and their force of elastic recoil is increased This increased force is reflected in a fall in intrapleural pressure that is the intrapleural pressure becomes increasingly subatmospheric as lung volume increases At the end of inspiration the inspiratory muscles relax and the lungs and the chest wall are forced back to the equilibrium volume mainly by the elastic recoil of the lungs If expiration continues further into the expiratory reserve compartment the expiratory muscles are increasingly called on to deform the thorax

The respiratory muscles encounter opposition of another sort They must supply the force necessary to produce flow of gas through the upper airway and the tracheobronchial tree and additional force to produce what might be called flow of the lung parenchyma and the moving tissues of the thoracic wall During quiet breathing the respiratory muscles need supply these forces only during inspiration the elastic recoil of the lungs is sufficient to overcome gas and tissue resistance during ordinary expiration

Just as the force required to oppose the elastic recoil of the lungs is reflected in the intrapleural pressure so are the forces necessary to overcome gas and tissue resistance of the lungs and airways For example during a rapid inspiration the expanding chest wall may be visualized as pulling on the lung surfaces and the lungs resist the pull not only with their own elastic recoil but also with the flow resistance of the airways and the moving tissue As a result, intrapleural pressure drops further below atmospheric pressure than can be accounted for by the elastic recoil alone During a forced rapid expiration the elastic recoil of the lungs is insufficient to produce so rapid a volume change Due to the action of expiratory muscles the chest wall may be thought of as pushing on the pleural surface of the lungs and the intrapleural pressure may be driven well above atmospheric pressure

Thus the pressure fluctuations at the surface of the lungs are not simply a result of lung elasticity—they relate also to flow resistance This is true during quiet breathing as well as during forced breathing If we are to obtain

a measure of the elastic and flow resistive properties of the lungs we must have a means of separating the intrapleural pressure fluctuations into elastic and flow resistive components An understanding of this process is the most important step toward understanding the principles of respiratory mechanics

The features of this analysis may be stated as follows

1 The elastic component of intrapleural pressure depends on the degree of stretch of the lungs and hence on the volume of the lungs It is independent of the rate at which lung volume is changing

2 The flow resistive component of intrapleural pressure depends on the rate at which lung volume is changing and is essentially independent of lung volume per se

At two points during the respiratory cycle namely at the end of inspiration and the end of expiration there is no flow in the normal lung At these instants intrapleural pressure depends entirely on elastic forces In the interval between these instants the lungs have changed volume If we relate this volume change to the associated change of intrapleural pressure measured at the instants of no flow we obtain a quantitative description of the elastic behavior of the lungs over this range of volume Expressed as volume change per unit of pressure change for example in liters cm of water the result is termed the *pulmonary* compliance Expressed as the change in pressure per unit of volume change this relationship has been called the *elastic resistance* or the *elastance* of the lungs

Before we consider measurement of flow resistance it is useful to examine the adequacy of the measurement of pulmonary compliance as a description of the elastic properties of the lungs So far we have related *changes* in lung volume to changes in intrapleural pressure saying nothing about the absolute levels of lung volume or pressure difference between the air passages and the lung surfaces For a more complete description of the elastic behavior of the lungs it is necessary to know the relationship between lung volume and the elastic component of intrapleural pressure over a wide range of lung volume from complete collapse to maximal expansion Further it would be desirable to take into account the size of the lungs

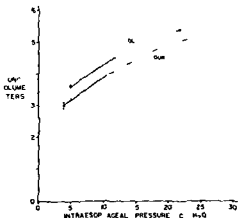


Fig 267 Graph showing relationships of lung volume and the elastic component of intrapleural pressure

that is the mass of lung tissue exclusive of the gas contained The importance of this factor may be illustrated by comparing compliance measurements made in two animal species of different body size for example the guinea pig and man The pulmonary compliance of an average man is 180 ml cm of water The pulmonary compliance of small guinea pigs is 0 ? ml cm of water roughly one thousandth that of man However when lung size is taken into account by expressing compliance per gram of lung tissue the lungs of the guinea pig and of man are found to be closely similar in their elastic behavior

It is not possible in practical measurements made during life to obtain accurate information about lung tissue mass or to study the elastic behavior in terms of absolute volume or pressure over a full range of lung expansion However it is possible to approach these ideals As an index of lung size we may take a measure of body size for example the height It is reassuring to note that measurements of pulmonary compliance made in infants children and adults vary approximately in the same ratio to body height as does lung weight

The lung volume change can be extended much beyond the resting tidal range voluntarily or by means of externally applied pressure as in a respirator Further the absolute volume of gas in the lungs can be measured by gas dilu

tion methods or more conveniently by the plethysmographic methods similar to those that have recently been developed in Dr Comroe s laboratory *It is the problem of measuring absolute intrapleural pressure safely and accurately that at present stands in the way of the ideal description of the elastic behavior in terms of absolute volume and pressure* The measurement of intra-esophageal pressure has provided a safe and convenient approximation of intrapleural pressure *change* as demonstrated by direct comparison of esophageal with intrapleural pressure However as yet the validity *of esophageal pressure as a measure of absolute* level of intrapleural pressure has not been established It appears that it offers a useful approximation at least in subjects studied in the upright position

An example of the usefulness of placing the relationships of lung volume and the elastic component of intrapleural pressure on an absolute scale is illustrated in Figure 262 The volume of gas in the lung is plotted on the ordinate against the intra-esophageal pressure relative to atmospheric pressure The two solid lines represent mean values obtained for a group of young subjects and a group of elderly healthy human subjects the subjects being matched for body height These lines represent the measurements made in the tidal range of lung volume The slopes of the lines are equivalent to compliance values that would be obtained during quiet breathing The slopes of the lines are nearly the same for the two groups the young and the old and indeed the difference in compliance is not statistically significant One might conclude from this much information that the lungs of elderly individuals behaved elastically in the same way as the lungs of young subjects This is not the case as may be seen when we compare the volumes and the pressures in absolute terms The functional residual capacities of the two groups of subjects were measured by a gas dilution method and all volume changes related to this volume When we compare the transpulmonary pressure at equal lung volumes we see that in all volumes the pressure is less in the older subjects Therefore we may conclude that in general the lungs of older individuals demonstrate a diminished elastic recoil compared with the lungs of younger individuals This finding is consistent with the observation that the functional residual capacity increases slightly with age

When we consider the measurement of flow resistive properties, it is important to note the shape of the elastic characteristics in the ordinary range of breathing In this range of volume a straight line relationship exists between the volume and the elastic component of transpulmonary pressure This fact is of use in isolating the flow resistive component of pressure for it may be seen that if we know the compliance of the lungs we can predict the elastic component pressure at any point during the respiratory cycle All we need to know is how much the volume has changed from the no flow point at either extreme of tidal volume Having predicted the elastic component at any instant during the respiratory cycle we can compare this pressure with the observed pressure at the same instant and the difference will represent the pressure overcoming flow resistance offered by the air passages and moving tissues

Knowledge of the principles of respiratory mechanics helps one to understand the pathologic *physiology of respiratory* poliomyelitis and the treatment of respiratory paralysis by any form of artificial respiration In the time remaining I shall be able to deal with only one aspect of respiratory mechanics namely the physical characteristics of a natural cough and attempts to simulate these events by mechanical devices

A natural cough (Fig 263) utilizes the respiratory muscles in such manner that a violent blast of air is forced out through a greatly narrowed tracheobronchial tree The mechanical advantage of the expiratory muscles is enhanced by a preliminary inspiratory effort While the glottis is closed expiratory effort begins and intrathoracic pressures build up to 80 or 100 ml of mercury The resistive pressure drop is indicated on the abscissa and the rate of flow in liters per minute on the ordinate As one begins to cough he takes in an inspiration then the pressure in the thorax is filled up to 80 or 100 ml of mercury *sometimes even higher* before the glottis is opened Since no volume change occurs in this phase except by simple compres-

sion there is no stress on the lung tissue In other words the alveolar and intrapleural pressures rise by almost the same amounts

When the glottis opens the tracheobronchial tree is almost instantly decompressed but the intrapleural pressure continues to rise in this case to over 100 ml of mercury We are ignoring in this figure the slight change in pressure due to change in lung volume The production of high intrapleural pressure tends to compress the tracheobronchial tree to produce a very high resistance the lumen of the tracheobronchial tree remaining at approximately atmospheric pressure In a vigorous cough the airway behaves almost like a critical orifice the flow being almost constant in the face of a high driving pressure This is almost a constant flow during the later part of the cough The resistance is actually decreasing rapidly toward the end of the cough For comparison the curves in the dotted lines are the volume flow pressure relationships of a forced expiration

In normal subjects the sustained expiratory flow may be 5 liters second or more but in patients with pulmonary disease such as polio the flow is much lower The effect of the expiratory flow rate on movement of secretions has not been examined quantitatively Ross and his associates at the University of Rochester have made the following computations based on their study of cough dynamics If the tracheal cross section is 1 5 cm during normal expiration the highest velocity of gas in the trachea will be over 6 meters second that is (15 m p h) at 7 liters second the corresponding velocity would be 46 meters second (100 m p h) If the tracheal compression during cough is taken into account the comparable velocity in the trachea will approach sonic speed Further since the kinetic energy of a moving gas stream increases as the square of the velocity the force available to move secretions in the compressed trachea would be 25 times greater than in the uncompressed trachea These workers concluded

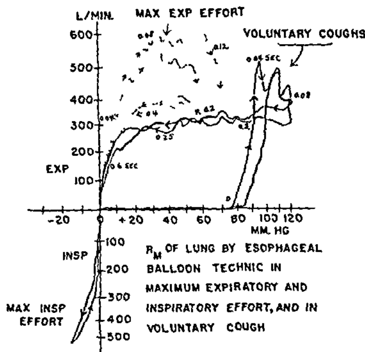


Fig 263 R_M of lung by esophageal balloon technic

that the compression of the tracheobronchial tree was as important to the objective of the cough as the generation of high flow rates

Several machines have been developed to imitate a cough in patients with weakened respiratory muscles These machines are to a variable extent successful in generating a high expiratory flow rate Figure 264 shows first a normal cough in which the initial pressure was over 120 ml of mercury the pattern of resistance change which relates to the degree of compression of the tracheobronchial tree and the curve for one of the cough simulating devices available in the United States It is obvious that these curves are very different The apparatuses, in general do not generate a high resistance in the lungs and one may infer that there is little or no compression of the trachea Therefore, such machines do not imitate one of the important characteristics of a cough

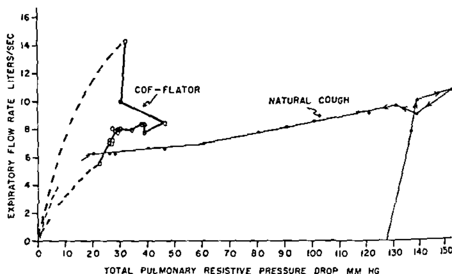


Fig 264 Illustration of natural cough and imitative cough generated by a cough machine

Evaluation of Respiratory Mechanics in Patients with Respiratory Muscle Paralysis

DR JERE MEAD

Dr Whittenberger has presented the principles of respiratory mechanics and has applied these principles in explaining the cough mechanism I should like to extend the application of these principles to the evaluation of the respiratory mechanical status of patients with respiratory muscle paralysis

In the patient with respiratory muscle paralysis respiration is accomplished by means of devices which apply pressure to the respiratory system It is a simple matter to measure the amplitude of the pressures applied the number of pressure cycles per minute and the ventilation accomplished This much information will suffice to permit useful deduction as to the mechanical properties of the patient s respiratory system

Dr Whittenberger has said that two physical properties govern the relationships between applied pressure and ventilation These are first the elastic recoil of the lungs and the chest wall and second the flow resistance offered by the air passages and the moving tissue It will simplify matters if we take up these two physical properties separately

If all we had to consider were the elastic properties of the lungs and the chest wall we could state quite simply the relationship between the frequency and the amplitude of applied pressure and the minute ventilation of the lungs In such a case the tidal volume would be directly proportional to the pressure amplitude within fairly broad limits Doubling the amplitude of pressure from 8 cm H_2O to 16 cm H_2O would nearly double the tidal volume Redoubling the pressure amplitude from 16 to 32 cm H_2O in general would not redouble the tidal volume at such large tidal volumes the elastic limits of the lungs and the chest wall would be approached and the compliance would be effectively reduced However in general tidal volume would be proportional to pressure amplitude in a purely elastic system Furthermore in a purely elastic system tidal volume would be independent of the cycling rate itself It would not matter how rapidly the pressure changed As long as flow resistance were absent the volume would be in phase with pressure and at a fixed amplitude of pressure tidal volume would not decrease as the cycling frequency increased Thus in a purely elastic system the tidal volume is directly proportional to the amplitude of applied pressure and is independent of the cycling rate

The relationship between applied pressure amplitude and cycling rate and the resulting tidal volume is not as simple as in the purely elastic system In this instance we will find it useful to concentrate on the minute ventilation that is the product of the tidal volume and the cycling rate rather than on the tidal volume itself The minute ventilation expresses the average rate of flow of gas for example in liters per minute passing into *and* out of the lungs The average rate of flow or minute ventilation is the same in either direction This would be assuming equal timing for the two phases of respiration for simplicity In a pure flow resistance system the minute ventilation depends on the average driving pressure in either direction The average driving pressure is related to the amplitude of pressure Thus we would predict that in a pure flow resistive system the minute ventilation will vary directly with the amplitude of applied pressure What can we say about the influence of the cycling rate? The average driving pressure during either phase of respiration will be the same for a given amplitude of pressure no matter how frequently the pressure is cycled Therefore minute ventilation will be independent of the cycling rate in this hypothetical case If this is so the tidal volume must decrease as the cycling rate is increased since the minute ventilation is

the product of the cycling rate and the tidal volume

We can now summarize our consideration of these two hypothetical cases In a purely elastic system tidal volume is proportional to the amplitude of pressure and remains the same as the cycling rate is increased In a flow resistive system tidal volume is proportional to the amplitude of pressure but decreases as the cycling rate is increased In the first case at a fixed amplitude of pressure minute ventilation will increase in proportion to the cycling rate In the second case minute ventilation will remain the same as the cycling rate is increased

If we now apply to our hypothetical systems the *concept of effective or alveolar ventilation* as developed by Dr Comroe in his presentation it becomes apparent that increases in cycling frequency at a fixed pressure amplitude will lead to a progressive reduction in *effective* ventilation if the impedance to ventilation is primarily flow resistive For if the minute *ventilation is nearly constant and hence tidal* volume inversely proportional to cycling frequency the dead space which represents a more or less fixed and ineffective portion of each tidal volume will receive an increasing proportion of the total ventilation as frequency is increased The remaining effective part the alveolar ventilation *will be reduced accordingly*

On the other hand if the impedance to ventilation is primarily elastic since the tidal volume will be maintained as the cycling rate is increased the effective portion of each tidal volume will tend to remain the same and effective ventilation will increase as the cycling rate is increased

Now let us apply these considerations to real rather than hypothetical examples The patient lies somewhere between the extremes we have examined He may approach one or the other of these extremes if he develops intercurrent pathology in his lungs or chest wall such as Dr Comroe and Dr Whittenberger have discussed We can now employ the relationships between frequency and tidal volume to obtain information as to the nature of his difficulty and to help us predict the most efficient pattern of pressure with which to assist ventilation

The equipment necessary is quite simple We will need some means of recording ventilation for example a low resistance spirometer or a low resistance valve system and a gas collection bag I emphasize the importance of low resistance in the recording equipment because the respirator must supply the pressures necessary to move gas through the measuring devices as well as gas in and out of the patient If these pressures are appreciable the result that we obtain will be more a measure of the mechanical properties of the measuring equipment than it will be of the patient The simplest means of measurement consists of a low resistance valve system and a gas collection bag If the resistance of the valve and bag system is less than 1 cm H O for a flow of 1 liter per second it will be *adequate for our needs by and large*

The range of adjustment of pressure amplitude and cycling rate for the particular respirator in use is also of importance to us in these measurements Fortunately the requirements are not severe If we can make a twofold change in cycling rate and at the same time adjust the *pressure amplitude so that it is approximately* equal at the two frequencies our present needs will be met and this is within the range of adjustment of most apparatus

Let us measure the tidal volume at two frequencies for example at 10 cycles per minute and 20 cycles per minute taking care to maintain *the pressure amplitude the same If the* tidal volume is reduced only slightly we can be sure that the principal mechanical impedance to ventilation is elastic in nature On the other hand if we find that the tidal volume is nearly cut in half when the frequency is doubled we can conclude that the mechanical impedance is *principally flow resistive If we have made* some measurements prior to the development of intercurrent pathology and know how much tidal volume decreases when cycling rate is doubled at the same pressure amplitude we can interpret changes in these measurements in terms of underlying mechanical abnormalities For example *complete obstruction of a main bronchus* will result in a substantial reduction in compliance Gastric distention will also reduce compliance Such patients would show little change in tidal volume with increased cycling rates On the other hand the increased flow resistance resulting from partial obstruction of the airway or from bronchoconstriction would *result in a pro-*

gressive reduction of tidal volume with increased cycling rate.

Now that we have developed simple means of testing whether the mechanical impedance to ventilation is primarily elastic or flow-resistive, we can consider the factors determining the optimal pattern of applied pressure to be used in a given circumstance. We will consider the several attributes of the pressure pattern separately. In each instance we will assume that the other attributes of the pattern are held constant. We will first take up the implication of the rate of cycling. Later we will consider the amplitude of pressure, the mean level of pressure, the shape of the pressure pattern and the timing of the phases of respiration.

The influence of the rate of cycling per se is implicit in the material that has been covered. If the principal mechanical impedance to ventilation is elastic, then, since tidal volume tends to be maintained at the same level as the frequency of cycling is increased, effective ventilation will increase more or less in direct proportion to the rate of cycling. If the principal impedance is flow-resistive, tidal volume will be inversely proportional to the rate of cycling, and although total ventilation will be little affected, the effective portion of ventilation that is alveolar ventilation will decrease as the cycling rate is increased. Since in no instance can the mechanical impedance be entirely flow-resistive, it does not follow that the optimal frequency for a patient with high flow resistance is the lowest frequency possible. In such a patient the tidal volume will indeed increase as the cycling rate is reduced, but a point will be reached when the elastic properties of the lungs and the chest wall limit further increases in tidal volume. The practical implications are these: Increasing the rate of a respirator will be detrimental rather than helpful if the principal difficulty is high flow resistance. Optimal rates in such circumstances will be in the neighborhood of 10 cycles per minute. At lower frequencies elastic properties will predominate in determining tidal volume even in instances where flow resistance is increased as much as tenfold above normal levels.

On the other hand, increasing the amplitude of pressure will increase the effective ventilation whether the mechanical difficulty be primarily elastic or flow-resistive in character. In either case tidal volume will increase roughly in direct proportion to pressure amplitude until the range of volume change approaches the elastic limit imposed by the lungs and the chest wall.

In the supine individual the range of volume change from the relaxed mid-position over which the compliance of the lungs and the thorax is approximately constant extends much further in the inspiratory direction than in the expiratory direction. This leads us to the question of the mean pressure level of the pressure cycle. It may readily be seen that as the amplitude of pressure is increased around a mean of atmospheric pressure, the elastic limit in the expiratory direction is approached before that in the inspiratory direction. To take advantage of this the most compliant portion of the volume-pressure curve, mean pressure must be increased in the airway relative to the body surface. As a result of this the mid-position will be increased, and this will be favorable in terms of flow resistance as well, since the flow resistance of the tracheobronchial system decreases as the average lung volume increases. However, the most favorable mean pressure in terms of respiratory mechanics may prejudice venous return to the heart, and the optimal mean pressure is a compromise between circulatory and respiratory effects.

In addition to the cycling frequency, the amplitude, the mean level of pressure, we must include the implications of the shape of the pressure pattern and the timing of the phases of respiration. I should like to put my emphasis on the importance of the timing of the pressure patterns. It could be regarded as being of more significance than merely the shape of the pattern per se.

Apart from questions of comfort, two important factors argue for reducing the time of the inspiratory phase relative to the expiratory phase in the pressure cycle. When flow resistance is elevated because of bronchoconstriction or partial obstruction of the small passages within the lungs, expiratory flow resistance tends to be greater than inspiratory flow resistance. This results from forces tending to collapse the air passages such as described by Dr. Wittenberger proximal to the regions of increased re-

sistance Such collapse can be minimized by reducing the mean driving pressure during expiration and prolonging the expiratory relative to the inspiratory phase A second advantage accrues from this to the extent that mean airway pressure is reduced thus minimizing the detrimental influence of pressure on cardiac return

I have made no attempt to survey the methods of measurement available or the accomplishments in research in the field of respiratory mechanics Simple methods have much to offer both in term of results and in terms of your way of thinking and the results obtained can be of use in the management of the patient who requires mechanical assistance to ventilation

Dr Heemstra Dr Comroe has explained that an adequate ventilation of functioning alveoli is one of the main requirements for pulmonary gas exchange Ventilation as such is a mechanical event and it is logical to expect that a knowledge of the mechanical behavior of the lungs and the thorax will help in understanding disturbances in the magnitude and the efficiency of ventilation and circulation in the lungs

As Dr Mead has told us relatively simple measurements can be very useful in determining whether the impedance to ventilation is primarily elastic or resistive in nature Such measurements do allow certain conclusions regarding the optimal rate and pattern of pressure variations during artificial ventilation

There is one point which has been already emphasized by Dr Whittenberger that may deserve further consideration the question of the description of the elastic behavior of the thorax and the lungs in terms of absolute lung volume and of absolute pleural pressure The choice of the level of respiration or in other words the expiratory lung volume during artificial ventilation is always to some extent a compromise between the most favorable conditions for the ventilation on the one hand and for the circulation on the other

From the ventilatory point of view a higher lung volume associated with a higher mean pressure level may be advantageous because both the total compliance of the thorax-lungs system is higher in this range and the flow resistance especially during expiration is lower

From the circulatory point of view high inflation pressures may cause a reduction in pulmonary blood flow both by their effect on venous return and by increasing pulmonary vascular resistance in part or all of the vascular bed This effect which is usually well compensated for in states of an adequate circulation may become serious in states of poor vasomotor reactivity low blood flow and circulatory inadequacy whether they result from bulbar involvement in poliomyelitis disease or from hypoxia or acid base derangement

It has been reported that in such cases a reduction of the mean pressure level may be advantageous Also a slight head-low position may increase the circulation in poliomyelitis patients However the question is how far can we go in reducing the expiratory level of ventilation in severely paralyzed patients? In a supine position as Dr Whittenberger has remarked the weight of the abdominal contents pushes the diaphragm upward especially when it is paralyzed This may be associated with a reduction in the functional residual capacity According to Mjorner and Svanberg the functional residual capacity of artificially ventilated polio patients is reduced from 1 65 to 1 30 liters in the supine position If a negative pressure phase is added a further reduction to 1 04 liters occurs

I would like to show here some of our own results with regard to the elastic behavior of the chest and the diaphragm and of the lungs in anesthetized normal and curarized rabbits (Fig 265) The resting expiratory level was varied by shifting the tracheal pressure to the positive or to the negative side The pleural pressure was measured directly and the volume changes of the animal were measured by a plethysmographic technic Curarization combined with intermittent artificial inflation did lower the breathing level in this case but the phenomenon which I like to emphasize is the shape and the range of the pressure-volume curves When the resting lung volume is reduced by the application of negative pressure to actively breathing rabbits most pressure difference is taken up by the chest and the diaphragm whereas in the paralyzed rabbits the pressure difference is taken up by the lungs From a certain level onward the lungs of the curarized animals are apparently unable to diminish further in size not because they are empty of air but because the conducting airways are collapsed Here the lungs prevent a further decrease in volume of the passive chest However in the active state increasingly stronger negative pressures are required to reduce the volume of the chest to low values This is

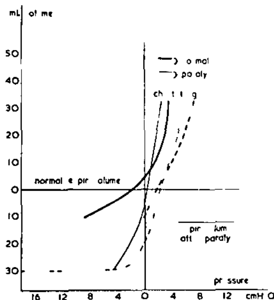


Fig 265 End-expiratory pressure volume relationships of the chest-cage (including diaphragm) and the lungs during spontaneous breathing and after muscular paralysis Succinylcholine instead of tubocurarine has been used to avoid an eventual bronchoconstriction caused by a histamine liberating action of curare The general conclusions remain the same

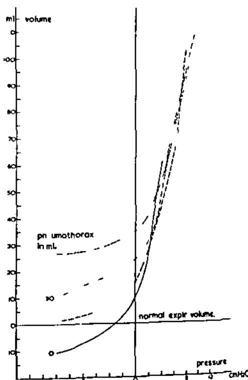


Fig 266 Chest wall pressure volume relationships in rabbits (means of / expts) at different lung volumes

caused by the development of an active inspiratory tone which is still present in the state of expiration Here the basic inspiratory tone of the chest and the diaphragm prevents the lungs from collapsing the airways remain open even on application of considerable negative pressure

We may conclude that the respiratory level especially when the lung volume tends to become smaller than normal is not simply the result of passive mechanical factors but that an active inspiratory tone of the chest musculature and diaphragm intervenes This active tone determines the shape of the chest compliance curve in normal animals It is a reflex tone brought about primarily by afferent impulses of collapse receptors in the lungs and mediated by afferent fibers in the vagus nerves I shall show you in Figure 266 a number of chest compliance curves obtained at varying degrees of pneumothorax Zero pneumothorax means pneumothorax of minimum size necessary to measure intrapleural pressure Compliance curves of the chest are shown with a pneumothorax of 15 30 and 45 cu cm so you see the only thing which is different in this case is the volume of the lungs The volume of the lungs determines the compliance curve of the chest

In paralyzed patients the lungs may be deprived of such a reflex protection preventing a too great reduction of the expiratory lung volume and a collapse of the *conducting airways*. When the application of a negative pressure phase in patients with an already reduced respiratory level would indeed lead to such a collapse there is little doubt that regions of atelectasis and a pronounced unequal distribution of ventilation would develop

A too great reduction of lung volume is perhaps also a danger in the application of mechanical *coughing devices* when the duration of the strongly negative phase is chosen too long Theoretically I cannot see why such coughing devices should not be able to create a redu tion in size of the intrathoracic air passages by creat

ing a pressure gradient across the bronchial wall as in a natural cough However it does seem possible that a collapse of the extrathoraci trachea could be produced also and that this would reduce the expiratory flow to such an extent that a narrowing of the intrathoracic airways would not occur

Summarizing we have seen now that there is a level of optimal complian e and resistance where a given pressure change will cause a maximal tidal volume There also exists a level where the conditions for the circulation are optimal and here one should be aware of the danger of a too low expiratory volume where the airways will collapse and regional atelectasis will follow

In considering the optimal level of re pira ion there is still another point which might be con sidered the efficiency of a ventilation of given size This efficiency depends not only on the anatomic dead spa e but also on the equality of the distribution of fresh air and blood to differ ent regions of the lungs This again is to some extent a mechanical problem and I should like to dis uss the question whether or not the effi ciency of ventilation normally depends on active muscular factors which are absent in passively ventilate l paralyzed patients

The lungs are a structure attached at the hilus to a more or less fixed point in the thora i cavity The exa t position of the lung hilus depends on the tension in the mediastinal con nective tissue which extends to the peri ardium and the diaphragm and on the other hand on the longitudinal tension in the tra hea which is governed by the position of the larynx From the hilus a framework of connective tissue radi ates into the periphery of the lungs and atta hes to the pleura The mechani al tension in this connective frames ork creates the retractive force This force is not necessarily the same at all regions of the pleural surface It depends on the position of the lung hilus on the intrinsic chara teristi s of the lung tissue and on the shape of the thoracic cavity There is now sufficient evidence for the existen e of local dif ferences in pleural pressure even in normal lungs What we measure as esophageal pres sure is probably near the pressure in the medias tinal or medial pleural spa e This pres ure at least in dogs is less negative than that in the

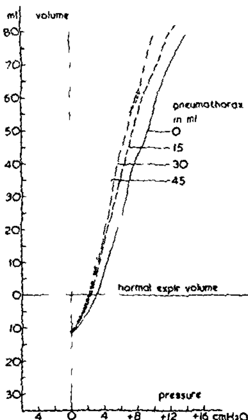


Fig 767 Lung-compliance curves in rabbits (means of 6 expts) measured at different pneumothorax volumes

lateral pleural space The matter of regional inequality of pleural pressure can be understood more easily if we realize that the free shape of the expanding lungs is not perfectly adapted to the shape of the chest in all phases of respira tion The degree of maladaptation determines the degree of pressure inequality Maladaptation also increases the pressure necessary to a hieve a given increase in volume but the effect on the distrib ution may be the more important one If a spherical rubber balloon is inflated within a cubi box more pressure is needed than when it is infla ed in a spherical box of the same content If the balloon contained a spongelike inner structure also the distribution of the inflated air would be quite different in both cases.

Figure 7" shows the results of lung-compli ance measurements in rabbits obtained during

pressure breathing with and without a pneumothorax It can be seen that a compliance is larger in the presence of a large pneumothorax when the lungs are not forced into the shape of *the adjoining chest wall*

Similarly changes in lung and thorax compliance have been observed by several authors after muscular paralysis Apart from other causes such as the accumulation of secretions and regional atelectasis it would seem that this is at least in part due to differences in the inflation mechanism in the active and the passive state Relatively slight differences in compliance when measured at an absolute level might be associated with relatively large changes in the distribution of ventilation

The mechanical situation which I have tried to describe is compatible with the hypothesis of a co-ordination of respiratory muscular activity in normal subjects in such a way that a good efficiency of ventilation is achieved In paralyzed subjects such a co-ordination may be deficient This would explain to some degree the reduction of mechanical compliance and the decrease in efficiency of ventilation occurring in such individuals

BIBLIOGRAPHY

Berneus B Gordh T Linderholm H Strom G and Werneman H Acta med scandinav 152 31 1955

Howell J B L and Peckett B W J Physiol 136 1 1957

Maloney J Jr and Handford S W J Appl Physiol 6 453 1954

Miorner C and Svanberg L Nord med 54 1818 1955

Dr Collier If we are to treat a patient in trouble with abnormal CO and or O tensions should we increase the ventilation or give oxygen or should we do both? These are the prime questions that we have to answer

Figure 268 is a diagram that we have found particularly useful in answering these questions If we take point A as representing the alveolar oxygen and carbon dioxide tensions of a patient who is in trouble the alveolar carbon dioxide tension is 80 mm of mercury with a consequently reduced alveolar oxygen tension of approximately 50 mm of mercury This patient we might choose to treat only by administration of oxygen If we added 20 per cent oxygen to the air which this patient was breathing this is a total of 37 per cent oxygen in the inspired air then the patient would move to point B This would be good treatment for the hypoxia of *point A but has done nothing to relieve the* respiratory acidosis that the patient would still have and the CO tension will remain at 80 mm of mercury A much more rational method of treatment for this patient would be to double approximately the alveolar ventilation and thus move the subject to point C which represents a carbon dioxide tension of approximately 40 mm of mercury and an alveolar oxygen tension of approximately 100 mm mercury thus tending to relieve both the respiratory acidosis and the lowered O tension

However the problem as Dr Comroe has pointed out is that point C represents only the alveolar tensions for both carbon dioxide and oxygen Problems of distribution or of diffusion lung pathology may cause the arterial-oxygen tension to be considerably below this value However if we double alveolar ventilation again we would increase the oxygen tension only to 125 mm of mercury in the alveolar gas and this would be at the expense of producing respiratory alkalosis A much more efficient procedure for a patient with abnormal lungs is to administer oxygen We can see that a marked relief of the hypoxia can be expected and this then would be without disturbing the acid-base balance of the body It is also interesting to note that on theoretical grounds Suskind and Rahn have pointed out that as CO tension goes below 40 mm of mercury the mixed venous O tension will actually decrease rather than increase so there may be little advantage in decreasing the CO tension below relatively normal values

Ideally then we feel that the respirator should be set to maintain the arterial or alveolar CO_2 tension and the arterial pH at normal values When this is done the arterial-oxygen tension and oxygen saturation will usually be satisfactory However if adequate blood oxygenation is not achieved because of lung pathology then oxygen should be administered in sufficient amounts to overcome the defect that is present Our studies on the arterial-oxygen tension in acute and chronic poliomyelitic patients indicate that while the alveolar arterial oxygen tension

difference is frequently greater than normal it is rarely necessary to add oxygen to the inspired air if the alveolar CO_2 tension is kept at normal values.

We have found that the alveolar air analysis with the infrared CO_2 analyzer has been of great value in the care of patients because we can rapidly evaluate the ventilatory status regardless of the manner of breathing, whether with positive pressure cuirass, the rocking bed, the abdominal pressure belt, glossopharyngeal breathing or breathing with the ordinary muscles. There is no apparatus dead space and the test requires no co-operation of the patient. The results are immediately available from breath to breath and the arterial CO_2 tensions can be estimated with accuracy in poliomyelitic patients by this means. There is one word of caution in depending too much on the arterial or alveolar carbon-dioxide tension. It is only one of the three unknowns in the Henderson Hasselbalch equation. In order to have a full understanding of the acid base balance of a given patient one must know either the pH or the serum CO_2 content as well. However, the presence of a metabolic acidosis or alkalosis frequently can be ruled out on clinical grounds alone so that we need do these additional studies only in questionable cases that are in trouble.

Some time ago we have used an automatic end tidal sampling method for obtaining alveolar gas for analysis. However, in respirator patients we frequently observed very poor results which we felt were due to the fact that some patients may have very widely variable tidal volumes due to swallowing or due to glottic [illegible] during artificial respiration.

However, in patients with heart or lung disease the methods of direct alveolar gas analysis cannot be used. We have found the end tidal CO_2 tension in some cases to be as much as 30 mm mercury below the arterial tension in patients with emphysema. This discrepancy of course is caused primarily by uneven distribution between the gas and the blood in the lungs in these patients.

It is fortunate that Dr Hackney working at the Los Angeles General Hospital has worked out a rebreathing method using the method of Christensen, Douglas and Haldane which permits an indirect estimation of the arterial CO_2 tension even in this group of patients. A demonstration of the infrared analyzer in the rebreathing method can be seen in the exhibit section in the other building. It may be possible to adapt this technic so that a simple chemical analysis may be used and render the infrared analyzer unnecessary.

The arterial or alveolar CO_2 tension should not be used as a guide for placing the patient in the respirator. Whenever it is possible artificial respiration should be instituted early enough to prevent rather than to treat hypoventilation.

In the absence of the methods of CO_2 analysis we have found that Radford's nomogram is quite satisfactory as a practical guide for setting the ventilation for the determination of the tidal volume. Ordinarily one can use any basal metabolism apparatus that one can find in an average hospital. However, a metabolism machine cannot be used readily when positive pressure is being applied to the airway and CO_2 analysis is almost essential in this circumstance.

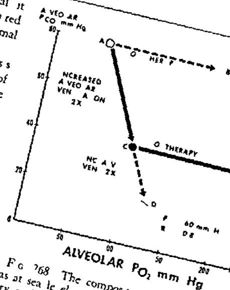


Fig. 268. The composition of alveolar gas at sea level in a steady state. Respiratory exchange ratio = 0.8. Points A, C and D show the relationship between alveolar P_{CO_2} and P_{O_2} while breathing room air (21% O_2). Points B and E show the relationship between alveolar P_{CO_2} and P_{O_2} while breathing 80 per cent air plus 20 per cent oxygen (37% O_2).

pressure breathing with and without a pneumothorax It can be seen that a compliance is larger in the presence of a large pneumothorax when the lungs are not forced into the shape of the adjoining chest wall

Similarly changes in lung and thorax compliance have been observed by several authors after muscular paralysis Apart from other causes such as the accumulation of secretions and regional atelectasis it would seem that this is at least in part due to differences in the inflation mechanism in the active and the passive state Relatively slight differences in compliance when measured at an absolute level might be associated with relatively large changes in the distribution of ventilation

The mechanical situation which I have tried to describe is compatible with the hypothesis of a co-ordination of respiratory muscular activity in normal subjects in such a way that a good efficiency of ventilation is achieved In paralyzed subjects such a co-ordination may be deficient This would explain to some degree the reduction of mechanical compliance and the decrease in efficiency of ventilation occurring in such individuals

BIBLIOGRAPHY

Berneus B Gordh T Linderholm H Strom G and Werneman H Acta med scandinav 152 31 1955

Howell J B L and Peckett B W J Physiol 136 1 1957

Maloney J Jr and Handford S W J Appl Physiol *6 453 1954*

Miorner G and Svanberg L Nord med 54 1818 1955

Dr Collier If we are to treat a patient in *trouble with* abnormal CO_2 *and* or O_2 *tensions* should we increase the ventilation or give oxygen or should we do both? These are the prime questions that we have to answer

Figure 268 is a diagram that we have found particularly useful in answering these questions If we take point A as representing the alveolar oxygen and carbon dioxide tensions of a patient who is in trouble the alveolar carbon dioxide tension is 80 mm of mercury with a consequently reduced alveolar oxygen tension of approximately 50 mm of mercury This patient we might choose to treat only by administration of oxygen If we added 20 per cent oxygen to the air which this patient was breathing this is a total of 37 per cent oxygen in the inspired air *then the patient would move to point B* This would be good treatment for the hypoxia of point A but has done nothing to relieve the respiratory acidosis that the patient would still have and the CO_2 tension will remain at 80 mm of mercury A much *more rational method* of treatment for this patient would be to double approximately the alveolar ventilation and thus move the subject to point C which represents a carbon dioxide tension of approximately 40 mm of mercury and an alveolar oxygen tension of approximately 100 mm mercury thus tending to relieve both the respiratory acidosis and the lowered O_2 tension

However the problem as Dr Comroe has pointed out is that point C represents only the alveolar tensions for both carbon dioxide and oxygen Problems of distribution or of diffusion lung pathology may cause the arterial-oxygen tension to be considerably below this value. However if we double alveolar ventilation again we would increase the oxygen tension only to 125 mm of mercury in the alveolar gas and this would be at the expense of producing respiratory alkalosis A much more efficient procedure for a patient with abnormal lungs is to administer oxygen We can see that a marked relief of the hypoxia can be expected and this then would be without disturbing the acid base balance of the body It is also interesting to note that on theoretical grounds Suskind and Rahn have pointed out that as CO_2 tension goes below 40 mm of mercury the mixed venous O_2 tension will actually decrease rather than increase so there may be little advantage in decreasing the CO_2 tension below relatively normal values

Ideally then we feel that the respirator should be set to maintain the arterial or alveolar CO_2 tension and the arterial pH at normal values When this is done the arterial-oxygen tension and oxygen saturation will *usually be* satisfactory However if adequate blood oxygenation is not achieved because of lung pathology then oxygen should be administered in sufficient amounts to overcome the defect that is present Our studies on the *arterial-oxygen* tension in acute and chronic poliomyelitic patients indicate that while the alveolar arterial oxygen tension

difference is frequently greater than normal it is rarely necessary to add oxygen to the inspired air if the alveolar CO tension is kept at normal values

We have found that the alveolar air analysis with the infrared CO analyzer has been of great value in the care of patients because we can rapidly evaluate the ventilatory status regardless of the manner of breathing whether with positive pressure cuirass the rocking bed the abdominal pressure belt glossopharyngeal breathing or breathing with the ordinary muscles There is no apparatus dead space and the test requires no co-operation of the patient The results are immediately available from breath to breath and the arterial CO tensions can b estimated with accuracy in poliomyelitic patients by this means There is one word of caution in depending too much on the arterial or alveolar carbon-dioxide tension It is only one of the three unknowns in the Henderson Hasselbalch equation In order to have a full understanding of the acid base balance of a given patient one must know either the pH or the serum CO content as well However the presence of a metabolic acidosis or alkalosis frequently can be ruled out on clinical grounds alone so that we need do these additional studies only in questionable cases that are in trouble

Some time ago we have used an automatic end tidal sampling method for obtaining alveolar gas for analysis However in respirator patients we frequently observed very poor results which we felt were due to the fact that some patients may have very widely variable tidal volumes due to swallowing or due to glottic valving during artificial respiration

However in patients with heart or lung disease the methods of direct alveolar gas analysis cannot be used. We have found the end tidal CO tension in some cases to be as much as 30 mm mercury below the arterial tension in patients with emphysema This discrepan y of course is caused primarily by uneven distribution between the gas and the blood in the lungs in these patients

It is fortunate that Dr Hackney working at the Los Angeles General Hospital has worked out a rebreathing method using the method of Christensen Douglas and Haldane whi h per

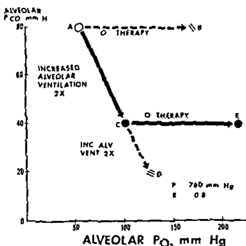


Fig 768 The composition of alveolar gas at sea level in a steady state Respiratory exchange ratio = 0 8 Points A C and D show the relationship between alveolar Pco and Po while breathing room air (?1 O) Points B and E show the relationship between alveolar Pco and Po while breathing 80 per cent air plus 70 per cent oxygen (37 O)

mits an indirect estimation of the arterial CO tension even in this group of patients A demonstration of the infrared analyzer in the rebreathing method an b n in the exhibit section in the other building It may be possible to adapt this technic so that a simple chemical analysis may be used and render the infrared analyzer unne essary

The arterial or al eolar CO t nsion should not be used as a guide for placing the patient in the respirator Whenever it is possible artificial respiration should be instituted early enough to prevent rather than to treat hypoventilation

In the absence of the methods of CO analysis we found that Radford s nomogram is quite sati factory as a practical guide for setting the ventilation for the determination of the tidal volume ordinarily one can use any basal metabolism apparatus that one can find in an average hospital Howe er a metabolism machine can not be used readily when positive pressure is being applied to the airway and CO analysis is almost essential in this circumstance

We feel that it is preferable to write a medical order for a given tidal volume and rate on a patient rather than to order a specific pressure setting In this manner we can use a pressure cycled respirator and retain many of the advantages of the volume-cycled respirator

Almost all of our patients with acute poliomyelitis are treated either in the tank respirator or in the tank respirator with added tracheal positive pressure and it is extremely rare that adequate ventilation cannot be achieved with this equipment In fact the majority of patients tend to be overventilated and we must exercise great vigilance if overventilation is to be prevented We have studied a number of patients with chronic respiratory paralysis and long standing hyperventilation In a selected group o 14 such patients the mean arterial Pco tension was 20 mm Hg and the mean arterial pH was 7 50 despite long standing hyperventilation The hyperventilation was corrected in all of these patients over a period of time by reducing the respirator pressures and in some cases by gradually transferring the patient to auxiliary respiratory aids such as the cuirass and the rocking bed A most striking thing in this study besides the abnormal blood chemistry was the marked change in psychological pattern that we observed By reducing the ventilation the patient was transformed from a complaining whining introspective individual into a patient with normal desires and interests outside of himself

One patient with zero vital capacity has been markedly hyperventilated in the tank respirator for 4 years requiring supplemental oxygen much of the time Repeated blood studies revealed an arterial Pco tension of 12 to 14 mm Hg However it was possible to reduce the ventilation gradually the oxygen was discontinued and the patient was transferred gradually to a cuirass and a rocking bed She was changed from a very severe nursing problem in the respirator center to a patient able to be cared for by her family at home

We had thought that perhaps much of the acquired dependence on an increased ventilation such as in this patient was due to the new setting of the respiratory center However certain other factors may be of importance In a small series Dr Crane of the Los Angeles General Hospital has observed that when the tank respirator is opened and the hyperventilated patient is allowed to breathe spontaneously the alveolar CO tension rapidly becomes normal When he is placed in the tank again the alveolor CO rapidly falls due to his subjective demand for *the excessive ventilation However when the* cuirass or the rocking bed is used the patients subjective ventilatory requirement is frequently markedly reduced from that required with the tank or positive pressure respiration We do not understand the reasons for these differences

In summary then we feel that extreme hyperventilation or hypoventilation are undesirable and we feel that we may prevent these only if we do have laboratory tests available to facilitate the institution of judicious treatment

Prof Mollaret I wish to speak of the circulatory disorders in poliomyelitis patients which it is necessary to be able to recognize although they are obscured by respiratory disorders This subject has become a major preoccupation of mine I will present some suggestive notes a coherent physiopathologic interpretation and finally an experimental treatment of startling audacity for cases of poliomyelitis in imminent danger of death

The first statement is that the study of circulatory disorders should henceforward take precedence over that of respiratory disorders In fact the preceding communications suffice to show that theoretic knowledge in this field is already quite satisfactory in addition a certain degree of mastery has been won in the treatment of such respiratory insufficiencies although this is true only of those who following the example of Lassen have not remained in the iron-lung stage This is an apparatus which can save only a small number of poliomyelitis cases but can kill many

The second statement is that the essential circulatory problem is found in the group of poliomyelitis patients dying of circulatory disorders *despite the fact that correct respiratory equilibrium has been assured* If circulatory collapse (shock) was formerly frequent it was because the collapse was almost always *secondary to a circulatory imbalance* Correction of the latter has reduced considerably this first category of circulatory difficulties so true is this that in our center we have inverted the traditional findings looking to changes in pulse and arterial

blood pressure for danger signs of an incipient breakdown in respiratory equilibrium

The third statement is that the field of *circulatory disorders not associated with respiratory difficulties is not unequivocal* and that several factors should be distinguished We will take only the last of these as the subject of our paper

1 It may be a case of circulatory death from *heart failure* following cardiac arrest in this case there is nothing to be done but to open the thorax at once and to massage the heart directly At our center everything is kept in readiness for this procedure and the number of patients we have been able to study under these circumstances already totals about a dozen

2 It may be a case of circulatory death from primary collapse (shock) we are incessantly alert for the major premonitory signs namely a combination of slowing of the pulse and a rise in arterial pressure in the complete absence of hypercapnia At this point cautious attempts can also be made to enlist the action of neuroplegic drugs At the critical stage of the disease marked by tachycardia and an abrupt drop in blood pressure recourse to neuroplegics gives the coup de grace and the most one can do is to try and retrieve the situation by continuous intravenous administration of noradrenalin (the beneficial effect of which will but too often wear off despite increasing dosage) We shall not dwell longer on this form of collapse of non respiratory origin merely contenting ourselves with adding that it may be associated either as a prelude or consequence with the third type of accident

3 Or again in fact it may be a case of circulatory death from *visceral hemorrhage* above all from gastro intestinal hemorrhage Such a hemorrhagic process ultimately will be fatal in all cases neither perfect respiratory equilibrium nor even the most extensive transfusions nor recourse to constant supportive chemotherapy will save such patients We wish here to describe understand and attempt to cure only this third type of circulatory complications in the light of investigations a preliminary account of which will be found in a recent paper written with various colleagues *

Consideration of these accidents is relatively recent and we have previously discussed the case reports of Heyde and Robinson (1948 2 cases) Erskine Mason and MacDade (1950 2 cases) Cook Hartmann Sarnoff and Berenberg (1951 7 cases) Rundlett (cited by Cook 7 cases) Lenarsky Parr and Seanor (1951 1 case) Baker Cornwell and Brown (1952 7 cases) Hoxsey (1953 5 cases) Schaberg Hildes and Alcock (1954 23 cases) and finally those of Bennike and Grandgeant (1956) In the papers mentioned above we ourselves have presented a detailed discussion of 11 cases to which we can add 3 new cases (exclusive of cases other than poliomyelitis but distinctly showing evidence of the same mechanism and amenable to the same treatment)

We will not repeat the clinical description noting only that 3 types may be distinguished (1) massive intractable hemorrhage fatal through acute anemia (2) moderate hemorrhage fatal through irreducible secondary collapse and (3) hemorrhage established only upon autopsy but having caused death before becoming externally manifest.

Instead we will briefly and very sketchily present our view of the histologic physiopathologic and therapeutic aspects involved

But first one comment is indispensable namely that it is necessary for any interpretation to account for 2 other series of phenomena of the gastro-intestinal tract which may coexist in poliomyelitis One of these is severe distention of the stomach and intestines which may have a very abrupt onset and likewise may be the forerunner of a fatal outcome the other is gastro intestinal ulceration the recognition of which has been comparable to that of hemorrhage Any adequate physiopathology must fit the explanations of the 3 phenomena into a coherent body of theory

HISTOLOGIC FINDINGS

Our histologic findings already seem to us to be very suggestive especially in regard to the unequivocal manner in which they indicate the existence of a common mechanism We are able to reproduce here only some of the slides shown at the meeting which dealt with all of our cases nevertheless enough illustrations are given here to make apparent the following signs of hemorrhagic process studied

* Re ne r 1 96 93 124 1957



F G ?69 Extens e d latat on of enules sl ght arter olar desquamat on



F G 2 0 Eno mous enous d la a on form ng a er able sponge









F G 271 (*Top left*) Typ cal ar er olar venous occlus on almost complete arter olar desquamat on (*Top* cal appe comple e and non nflamma o y om non nflammatory endothel al *left*) Vascular l d ssoci d apede c hemorrhage (*Bo om* Edema and d ssoc ascular stroma (*Bottom right*)

A First there are the direct vascular signs

1 *In the capillaries* congestion which might be considered unimportant

2 *In the venules* maximum vasodilatation far surpassing paralytic vasodilation in appearance (Figs 269 270 271 *top left and bottom left* and 272) the venous walls may show no more than slight pathology their involvement being confined to a more or less desquamated endothelium Thrombosis is absent or rare and if it exists is minimally inflammatory (no polynuclear cells)

3 *In the arterioles* considerable desquamation of the endothelium ranging from simple detachment (Figs 269 and 271 *bottom left*) to obstruction of the lumen (Fig 271 *top left*) here too the lesion is minimally inflammatory

B There are in addition indirect or paravascular signs

1 *Diapedetic hemorrhages* are possible sometimes becoming great oozing patches (Fig 271 *top right*)

2 *Edema of the submucosa* may be extensive detaching large stretches of the layer and seriously imperiling the nutrition of the tissues dependent on it (Fig 271 *bottom left and right*) we will only remark briefly that here we find the explanation of the genesis of those gastro-intestinal ulcerations whose importance we pointed out above

3 Finally there is *swelling and hyperplasia of the lymphoid structures* the hyperplasia also being noninflammatory Figure 272 shows a typical example

On the whole all our cases exhibited—in varying degrees but always unmistakably—such lesions all of which fall into the same category of completely noninflammatory but basically mechanical lesions This aspect was already known to us and we regard it as a specific type of severe vasomotor disturbance

PHYSIOPATHOLOGIC INTERPRETATION

The authors we have cited as reporting clinical cases have not failed to postulate explanatory mechanisms we list these but only to reject them all

1 *The role of respiratory disequilibrium* in particular that of anoxia (all of our patients exhibited normal respiratory equilibrium)

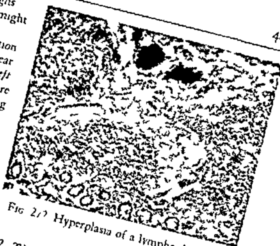


Fig 272 Hyperplasia of a lymphoid follicle

2 *The role of a stomach tube* (in our cases either it was not used or its use was discontinued)

3 *The role of blood dyscrasia* resulting from the poliomyelitis (this is the explanation of Bennike and Grandgeant we have never found any evidence of it)

4 *The role of direct visceral invasion* of the poliomyelitis virus (absence of any inflammatory process in the histology which is rather basically mechanical)

5 *The role of vasomotor paralysis* resulting from lesions of the vasomotor neurons of the brain stem the hemorrhagic syndrome thus becoming a deficiency syndrome as is paralysis of the extremities due to lesions of the neurons of the anterior medullary horns

To this list of explanations we oppose our own hypothesis which is that a vasomotor disturbance is indeed involved *not however a deficiency vasomotor disorder but rather an irritative one* It is not at all a question of a paralytic process but rather of an active sustained process This will be of capital importance from the therapeutic standpoint since the indication will be not to excite but to paralyze or better still to block the nociceptive maintenance circuits

Here again we can only present our argument in outline referring to the paper cited and to forthcoming publications for further details

1 *The histologic lesions* are exactly those described by J Reilly as occurring in laboratory stimulation of the sympathetic nervous system Mr Reilly's brilliant work since 1934 at the

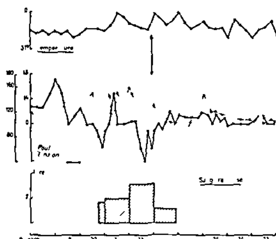


FIG 273 First case treated by bilateral profrontal procainization Immediate normalization of pulse and arterial tension (only the maximum pressure is shown)

Claude Bernard Hospital has elucidated this syndrome a bibliography of his works will be found in the paper mentioned before (see footnote on p 443)

2 *The histologic lesions and their progressive course* are duplicated in the events leading to the death of certain subjects of neurosurgery *especially in operations involving the posterior* cerebral fossa (Cushing De Martel Clovis Vincent etc)

3 *The histologic lesions and their progessive course* are analogous to those so often described in the pathology of infectious diseases by Hutinel s designation malign syndrome the predominant lesions are analogous to those seen in our poliomyelitis cases (with due allowances for the diffusion of the lesions)

4 In addition *the points of agreement* between such an irritative mechanism and the vascular and paravascular lesions observed are easily understood

(a) *the desquamation of the arterioles* reflects the initial state in which there is extensive vasoconstrictions

(b) *the venous lesions* are a sign of active vasodilatation and evidence the enormous accumulation of blood in a veritable enormous visceral sponge this accumulation drains the cardiac pump and engenders fatal secondary shock

5 *The two combinations mentioned* are equally easy to explain on the one hand the severe gastro-intestinal dilatation (concomitant parasympathetic involvement) and on the other the ulcerations of the digestive tract (due to nutritional disorders brought about by lesions of the submucosa)

6 We may look to *therapy* for possible confirmation, a question which we will take up in our final section

THERAPEUTIC TRIALS

Let us first recall very briefly the major finding stated at the outset of this paper the invariably fatal course of the hemorrhagic syndrome under investigation In particular all attempts at therapy aiming at stimulation of the vasomotor centers not only fail but hasten the process

Therefore when faced with another hopeless case we believed that we were warranted in attempting the inverse namely to aim at *paralyzing vasomotor activity* by direct pharmacodynamic blocking of the nociceptive maintenance circuits

Direct bifrontal instillation of procaine after double trepanation was our first attempt of this nature This was done for reasons detailed at length at the time particularly in order to act on the highest confluence of the neurovegetative system

The first case thus treated has been reported in detail in the paper cited before (footnote p 443) Although the patient died 47 days after the intervention (following a pulmonary infection due to *Pseudomonas aeruginosa* on the 26th day) the immediate benefit had been spectacular the charts in Figure 273 show the immediate and lasting normalization of pulse and arterial pressure The arrest of hemorrhage was also immediate (allowing for the time necessary for evacuation of blood accumulated in the digestive tract)

In a second case (still unpublished) death occurred as early as the 6th day but for other reasons and after the same immediate benefits had been noted The beneficial effects extended to a severe concomitant syndrome of dilatation of the digestive tract which receded almost instantaneously

Bilateral prefrontal procainization was not at

all our final measure we had also contemplated recourse to other methods.

Our second approach was also to attempt high neurovegetative saturation but *by the intraventricular route*. This method was based on parallel investigations on the dog carried out at the Claude Bernard Hospital by J. Tardieu and J. J. Pocidalo who acquainted us with the details of their experiments. We shall only remark that this research dealt with cardiac insufficiency in 36 dogs whose nerve centers had been treated with procaine either by ascending spinal anesthesia (21 animals) or by intraventricular injection following trepanation. In the latter case either with (10 animals) or without (5 animals) previous spinal anesthesia.

The positive findings of these experiments seemed to us to warrant a trial of such procainization in man. In fact the first patient whom we tried this technic prompted by his hopeless state was a neurologic patient but not a poliomyelitis case (unpublished). This was a case of quadriplegia secondary to an injury of the upper cervical vertebrae. An exploratory laminectomy resulted in no benefit and some days later a severe hemorrhagic syndrome set in associated with collapse and severe gastro-intestinal dilatation. An intraventricular injection of 0.5 per cent procaine had a remarkable effect on the abdominal distention but was unable to forestall death which occurred 48 hours later.

The third approach was much simpler. It was an attempt to sever the nociceptive circuits at the lowest level of the nervous system, i.e., the radicular, by intraspinal procainization. The injection was made via the lumbar route and the patient was subjected to very carefully controlled progressive inclination. The first case thus treated (unpublished) was again not one of poliomyelitis but a woman with extremely severe postabortion tetanus. The patient had been tracheotomized and curarized; the appearance on the fifth day of treatment of the severe neurovegetative disorder described induced us to administer a slow intraspinal injection of 34 cc. of 1 per cent procaine. The benefit was astonishing (unpublished case).

The next two cases were of severe poliomyelitis with the same neurovegetative syndrome. The same treatment was carried out and was also followed by immediate favorable results. We ourselves should like to stress that it is still too early to speak of the ultimate survival of these patients; however, this does not detract from the fact that there was an immediate reversal in the condition of these patients, both of whom had been despaired of.

We do not feel warranted at the present time in going beyond this statement. However, we believe that no problem calls more urgently for thorough study than that of circulatory disturbances in poliomyelitis patients without respiratory dysequilibrium. Perhaps the near future will bring confirmation of our working hypothesis from the physiopathologic as well as the therapeutic point of view.

Swallowing Mechanism and Its Disturbances

DR JAMES F BOSMA

Disability of swallow is one of the most critical patterns of involvement of the *acutely ill and* of the persistently impaired poliomyelitis patient The acute bulbar poliomyelitis patient with respiratory *distress as a result* of paralysis of the pharynx is a familiar clinical picture and one which deserves the most *immediate* therapeutic effort of the physician The extent of motor disability of swallow is maximal at the time of the acute illness of poliomyelitis and immediately thereafter Appropriately many subjects who have temporarily lost their swallow function during acute poliomyelitis recover it in the first days or weeks of convalescence However there is a progressive accumulation of postpoliomyelitic subjects who have residual disabilities of this performance

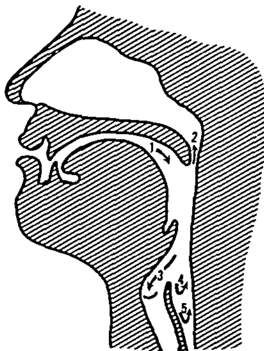


Fig 274 Poliomyelitic disability of the pharynx

A group of 34 patients child and adult having moderate or severe residual poliomyelitic disability of the pharynx have been observed by standardized clinical and roentgenographic procedures The particular pattern of their disabilities has proved to be markedly varied They may be pragmatically classified (Figure 274) as follows

1 Impairment of control of penetration of the bolus from the mouth into the pharynx

2 Abnormal penetration of the bolus up through this palatopharyngeal isthmus and into the nose

3 Abnormal penetration in the airway

4 An arrest at the level of the hypopharynx

5 An arrest at the level of the entrance to the esophagus

In Figure 275 we see a bolus being taken by the tongue from the oral pool and being carried up to its preparatory position here It is a function of the tongue to size the bolus correctly so that in successive swallows of material of light consistency the quantity collected by the tongue and sent down the pharynx is approximately similar [30] The bolus is carried by the tongue to a swallow preparatory position where it is held by the tongue firmly against the posterior portion of the palate until it is ready to be sent down as the swallow In order to hold the bolus there it is necessary that the tongue be of adequate volume that these muscles which suspend the tongue from the base of the cranium and from the mandible have a sufficient strength and also that the palate may be approximated toward the tongue [11] All of these are required for an adequate control of the bolus in the swallow preparatory position and to prevent its premature entrance in the pharynx

Figure 276 is a patient example in which two abnormalities of deglutition are shown On the left note the bolus spilling without control from the mouth into the pharynx In this patient





Fig 275 (*Top left*) Bolus being prepared for swallowing by the tongue

Fig 276 (*Top right*) Two abnormalities of deglutition

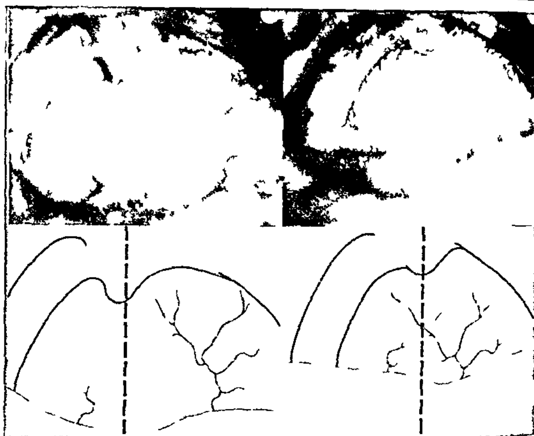


Fig 277 (*Bottom left*) Bolus enclosed normally within an indentation on the dorsal surface of the tongue

the reasons for this spill are (1) that the volume of the tongue is diminished (the dots on the illustration outline bolus which is penetrated into a trophic area of the tongue) and (2) the deficiency of suspensory mechanism of the tongue so the tongue cannot be held in position necessary to control this stream A patient having this disability performs an unusual maneuver during eating whereby he bends his head forward while he is masticating or preparing the material in the mouth and then he abruptly tips his head upward and backward to decant the bolus into the pharynx as an unquantified and unsized mass

The bolus as it penetrates into the pharynx (Fig 277) is enclosed normally within an indentation on the dorsal aspect of the tongue [6] At this time all other structures of the pharynx are raised and the palate is indented upward and backward The patient shown in Figure 276 also demonstrates the problem of nasal regurgitation In Figure 276 right the bolus now is in the pharynx and part of it is penetrating upward between the palatopharyngeal folds and the postpharyngeal wall in the palatopharyngeal isthmus The control of the palatopharyngeal isthmus depends on adequate motor strength of the palatopharyngeal muscles and also on adequate motor strength of the middle constrictor muscle of the pharynx which normally draws the lower border of the palatopharyngeal folds together Asymmetrical weakness of the middle constrictor muscle of the pharynx may be identified by inspection of the pharynx through the open mouth during phonation (Fig 278) In this example the middle constrictor muscle on the patient s left side is weak As we ask the patient to phonate we see that the posterior pharyngeal wall is drawn sharply toward his right (stronger) side This is the curtain movement of Vernet [9]

In the circumstance of slight weakness of the palatopharyngeal muscles the palatopharyngeal folds may be sufficiently drawn together so that abnormal penetration of the bolus upward into



Fi 2 8 Weak m ddle cons r ctor mu cle on the left s de of pharynx sho n dur ng phonat on

the nasal pharynx does not oc ur Ho ve er in the c rcumstance of v eakness of the muscles of ele at ng the pharynx generally there may be a fa lure of approx nat on of the palatopharyngeal folds vhen other v se h s approx mat on vould occur Th s veakness can be e aluated by plac ng the fingers of the exam ner n the groove bet veen the hyo d bone and the larynx (Fig 2 9) to observe the streng h of ele at on of the pharynx dur ng s valllo v There is also a veakness of closure of the palatopharyngeal folds so that there w ll be a leak up ard through the isthmus and pos bly a nasal regurgitat on of a part of the bolus

Contra ture of the sternohyo deus and sternothyro deus muscles is an abno mal ty of like effect to v eakness of the ele ators of the pharynx We can e aluate th s contracture by ha ng the subject extend h s head In F gure 280 ve see that in a normal subje t extend ng h s head the hyo d bone and all stru tu es rela ed to are carr ed up vard and rema n n the same relat e pos t on to the upper cer cal e eb ae (In th s roentgenogram the th rd cer cal e te bra is outl ned) In he c rcumstance of contracture of these muscles t s qu e appa ent ha the subject w ll suffer mpa rment of those funct ons of the pharynx h ch depend on s adequate up ard mot on

No as the bolus penetrates through h pharynx and do vn into the esophagus the s ru tures of the pharynx ele a e st ll further (F g 281) The palate is angled sharply up ard The tongue and the hyo d bone are al o at their max mal ele a on If there is weakness of he muscles suspend ng the s ructures of the an or pharyngeal wall from the base of the cran um these structures may be d splaced anter orly and the anteropos er or d ameter of the pharynx n crea ed (Fig 282) In these subjects the pr n

pal motion of the tongue the hyoid bone and the larynx during swallow may be forward or anteriorward instead of upward

The third possibility of abnormality of swallow is that of abnormal penetration of food into the larynx The larynx is closed in swallow by the apposition of the arytenoid cartilages in the midline and elevation of the thyroid cartilage so that the arytenoid[4 1 7] and false vocal cords[17 1] are brought to proximity with the undersurface of the epiglottis This arytenoid barrier to penetration of the bolus is not uniformly effective in normal subjects and by roentgen observation traces of bolus are at times seen to spill into the vestibule of the larynx though they do not penetrate through the false and true vocal cords to reach the trachea

In subjects with poliomyelitic impairment of the complex musculature of the anterior wall of the hypopharynx there may be specific impairment of convergence of the arytenoid cartilages and the true and false vocal folds Or there may be a deficiency of the more general elevation of the thyroid cartilage to accomplish *approximation of the arytenoids and structures* of the pharynx wall to the epiglottis A penetration of bolus into the larynx may occur in the face of relative adequacy of the arytenoid valve if there is obstruction at the pharyngoesophageal segment causing accumulation of bolus in the hypopharynx and its secondary overflow into the larynx

In the pharyngo-esophageal segment there are two distinct patterns of bolus obstruction These are

1 Occlusion of the hypopharynx by a crico pharyngeal bar formed apparently by contracture of the cricopharyngeus portion of the inferior constrictor muscle

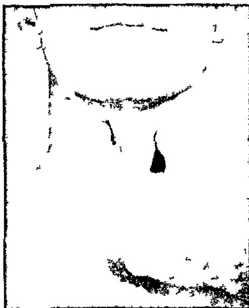


Fig 279 Use of fingers in evaluating the strength of elevation of the pharynx during swallowing



Fig 280 Elevation of pharynx structures when the head is extended in a normal subject.



Fig 281 Elevation of pharynx structures as bolus penetrates through the pharynx and down into the esophagus

2 Persistent closure of the sphincter at the orifice of the esophagus

The first of these patterns is found infrequently In our 34 subjects who were studied by reason of residual disabilities of pharynx following poliomyelitis we identified this roentgenographically in only three This roentgen demonstration is fairly difficult for the discrete bar of the cricopharyngeus portion of the inferior constrictor muscle is distinguishable but briefly during the swallow action protruding from the posterior pharyngeal wall after the first of the moving bolus has passed this level In Figure 283 are example frames taken from cinefluorographic studies showing this discrete cricopharyngeal bar protruding sharply from the posterior wall to approximate the anterior wall

The more common pattern of obstruction in this area is persistent closure of the circular sphincter at the orifice of the esophagus The motor mechanism of opening of the hypopharyngeal sphincter in the normal subject is not completely understood The sphincter is normally tightly closed and capable of resisting considerable increase in intrapharyngeal pressure Its opening undoubtedly reflects inhibition of its circular musculature It is noteworthy in this connection that in electromyographic studies of the musculature of the pharynx area in do



Fig 282 Increase in diameter of the pharynx due to anterior displacement of supporting muscles



Fig 283 Protrusion of discrete cricopharyngeal bar from posterior wall of pharynx

during swallow (Fig 284) a wave of inhibition was found to precede the sequential activation of the muscles in this sphincter area In the human according to Negus[18] and Antoni[3] the co-ordination of the hypopharyngeal sphincter involves distinguishable somatic and sympathetic motor elements the latter from the superior cervical ganglion The precise physiologic relation of the somatically and the sympathetically innervated musculature in the area of the sphincter of man requires further observation

It is apparent that in usual modes of swallow elevation of the larynx is essential to this part of the swallow performance With final elevation of the larynx as the bolus penetrates the hypopharynx the sphincter opens immediately and continuedly[9] An active mechanism of opening of the sphincter has been inferred[1, 10, 14, 8] from anatomic studies of Terracol and Nihet (Fig 285) in which a discrete midline bundle of muscle fibers was found to ascend from the anterior and the anterior lateral portion of the circular constrictor at the orifice of the esophagus to the posterior aspect of the cricoid cartilage and by careful dissections is found to send some fibers up toward the cartilages of Santorini Negus has pointed out a relation between closing of the larynx and opening of the orifice of the esophagus attributing this opening to forward motion of the cartilages of Santorini by the underlying interarytenoid muscle[18] This forward motion of the arytenoid cartilages has been observed by Ardran and Kemp[5] and also by ourselves in cinefluorographic studies of normal swallow However in poliomyelitic subjects having persistently closed hypopharyngeal sphincter the aditus may be well closed preventing penetration of the pooled bolus into the vestibule of the larynx Apparently then this vertical and sphincteric motion within the arytenoid area is not uniformly associated with opening of the hypopharyngeal sphincter in man

In the circumstance of persistent closure of this sphincter in 6 postpoliomyelitic impaired subjects the contour of the sphincter has been entirely normal and a catheter was found to penetrate in its midportion as in normals The sphincter remained firmly closed during maneuvers of air inflation of the pharynx or combined inflation of the pharynx and the esophagus after air had been instilled into the esophagus by catheter However others have reported success of such patients forcing food from hypopharynx into esophagus by air inflation of the pharynx[3] Our study subjects were unable to swallow fluids or a catheter but could readily accomplish penetration of a catheter by the procedure of gagging Appropriately they were able to vomit on occasion with apparently normal emesis stream Recovery of penetration through this orifice was most typically abrupt and roentgen examination of satisfactory swallows later demonstrated normal contour of the open sphincter Dilatation procedures are commonly ineffective in relieving this obstruction

The possible explanations for this pattern of obstruction are several including

1 *Deficiency of vertical traction from the*

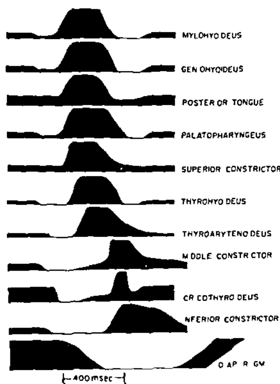


Fig 284 Musculature of the pharynx area in dog during swallowing



Fig 285 Midline bundle of muscle fiber (Terracol and Nichet)

hyoid the thyroid and cricoid cartilages (See above)

2 *Contracture or fibrosis of the circular musculature of the orifice of the esophagus* This could well occur in association with a generalized syndrome of myopathy such as dermatomyositis[1] or myoporphyria [10]

3 *Swallow inhibitory influences from the mucosa of the hypopharynx or of the adjacent esophagus* The hypopharynx commonly demonstrates chronic inflammation in association with stasis of secretions [19] Swallow inhibitory afferent effects may originate from chronically inflamed mucosae of pharynx or esophagus [7 19] It is of interest in this connection to note the exaggeration of gag reflex in these patients having complete incapacity of swallow With resumption of adequate swallow the gag reflex subsides to normal in both its readiness of elicitation and its degree of response There is no clear clinical evidence that the exaggeration of gag reflex and the absence of swallow are related phenomena though in neurophysiologic studies of patterns of motor co-ordination in the bulbar area as elicited by stimulation of the sup laryngeal nerve it was found that the perf ances of swallow and of emesis were mutu exclusive [3]

4 *A co ordinative impairment of the ac of swallow* resulting perhaps from impair of the swallow regulatory nuclear mechani Sjoberg [4] has mentioned as a sequel of p myelitis the clinical situation of recen demonstrated atony of the esophagus with traction in the upper esophageal orifice Th also in the lower esophageal orifice Th attributes to bilateral poliomyelitic ablatio vagal nuclei an effect essentially identica that which he had observed in experim vagal transection in the cat in confirmatio early experiments of Pavlov [6]

5 Functional disability in which dysph may be continued as an anatomically and p ologically inappropriate persistence of a disability

We have gained increasing respect for functional aspects of disability of swallow are now engaged in a study of severely

phagic patients obtaining history of early feeding in infancy and observing their general attitude toward food and their physiologic response to food applied to mouth or directly to lower areas of the alimentary tract. We find the evaluation of the functional elements of the total swallow-disabilities situation to be most difficult and complex

REFERENCES

1 Abel W The arrangement of the longitudinal and circular musculature at the upper end of the esophagus J Anat & Physiol 47 38 1912 1913
2 Anderson A C Lockhard R D and Souter W C Lateral syndrome of the medulla Brain 54 460 1931
3 Antoni N P E Uber Schlundlahmung Acta med scandinav 59 745 1923
4 Ardran C M and Kemp F H Closure and opening of the larynx during swallowing Brit J Radiol 29 205 1956
5 ——— The protection of the laryngal air way during swallowing Brit J Radiol 25 406 1952
6 ——— Radiographic study of the movement of the tongue in swallowing Dental Practitioner 5 252 1955
7 Bakay L In Kelemen G Rontgenuntersuchung der Mund—und Rachenhohle Berberich Lehrbuch der Mund und Rachen krankheiten Leipzig Thieme 1932
8 Baker A B Matzke H A and Brown J R Poliomyelitis III Bulbar poliomyelitis a study of medullary function Arch Neurol & Psychiat 63 257 1950
9 Barclay A E The practical importance of mechanics in digestion Am J Roentgenol 40 375 1938
10 Birmingham A A study of the arrangement of the muscular fibres at the upper end of the oesophagus J Anat & Physiol 33 10 1898
11 Bosma J F Studies of the pharynx I Poliomyelitic disabilities of the upper pharynx Pediatrics 19 881 1957
12 Czermak J N Gesammelte Schriften I 1858 As Reviewed in Eykman P H Der Schlingakt dargestellt nach Bewegungsphotographien mittelst Rontgen Strahlen Arch ges Physiol 99 513 1903
13 Doty R W and Bosma J F An electromyographic analysis of reflex deglutition J Neurophysiol 19 44 1956
14 Fishman L Z Laryngeal and esophageal atavism in man as indicated by the probable phylogenesis of the hypopharyngeal receptacle concerned in the act of deglutition Ann Otol Rhin & Laryng 44 139 1935
15 Lindsay J R Functional disturbances of the upper swallowing mechanism Ann Otol Rhin & Laryng 64 766 1955
16 Montandon A Pseudo spasme permenent de la bouche de l oesopnage et myoporphyria Pract oto-rhino laryng 10 267 1948
17 Negus V E Anatomic and physiologic considerations in paralysis of the larynx Proc Roy Soc Med 40 849 1947
18 ——— Mechanism of swallowing Proc Roy Soc Med 36 85 1942
19 Pannewitz V Partielle Schlu klahmung als Rontgensymptom bei Erkrankungen des Oesophagus Fortschr Geb Rontgenstrahlen 44 170 1931
20 Pavlov I P In Bechterew W Die Funktionen der Nervenzentra Berlin Fischer 1911
21 Pressman J J and Kelemen G Physiology of the larynx Physiol Rev 35 506 1955
22 Péthi L Der Schlingakt Sitz der Akademie der Wissensch 100 1891 (As reviewed in Eykman P H Der Schlingakt dargestellt nach Bewegungsphotographien mittels Rontgen Strahlen Arch ges Physiol 99 513 1903
23 Silverman F N Referred to in Bosma J F The mechanism of dysphagia in bulbar pharyngeal poliomyelitis A M A Am J Dis Child 86 637 1953
24 Sjoberg A Experimental and clinical studies of paresis of the pharynx larynx and esophagus Acta oto-laryng Supp 38 1939
25 ——— The suffocation symptomatology of bulbo-spinal poliomyelitis and experience of its operative treatment Acta oto-laryng Supp 95 p 291 1950
26 Stuart T P A On the mechanism of the closure of the larynx Proc Roy Soc London 50 232 1891 1892
27 Stuart T P A and McCormick A The position of the epiglottis in swallowing J Anat & Physiol 26 231 1892
28 Terracol J and Nichet L Le segment pharyngo-esophagus Rev Laryng 58 150 1937
29 Vernet M Le paralysie du glossopharyngien Le signe caracteristique de la paralysie du constricteur superieure du pharynx Paris Med 52 551 1916
30 Whillis J Movement of the tongue in swallow J Anat 80 115 1946

DISCUSSION

DR ARDRAN Dr Bosma has summarized the work that he and his colleagues have done relating to the disturbances of the swallowing mechanism following poliomyelitis and has given an extensive review of the literature With my colleague Dr F H Kemp and others we have investigated the problem on slightly different lines we have been concerned primarily with the behavior of the normal and its variations and have extended our work into the pathologic field only comparatively recently Our experience of the problems following poliomyelitis has been broadly the same as Dr Bosma s our criticisms of his work are primarily those of the interpretation of what is normal We have had the advantage of investigating hundreds of normal and abnormal individuals by cineradiography at 25 to 50 frames per second as well as by other methods Twenty four patients with swallowing defects following poliomyelitis have been studied It is important to obtain a full accurate history We have seen several patients who have been described as having some difficulty in swallowing following poliomyelitis but by careful inquiry have found that these symptoms have been due to conditions present before the onset of poliomyelitis resulting from accidents injuries or operations in the region of the neck The inability to hold fluid contrast medium in the mouth without it leaking into the pharynx and some degree of pharyngeal residue may be due to the results of previous tonsillectomy

Variations in the swallowing pattern may also be brought about by posture All normal individuals in the erect position swallowing a fluid bolus approximate the anterior and the posterior pharyngeal walls and completely strip the contents into the esophagus The epiglottis tilts backward as the larynx is elevated and the tongue of the epiglottis turns over the entrance of the larynx as the last of the bolus is stripped from the pharynx However many individuals when swallowing with the head extended on the neck are unable completely to approximate the anterior and the posterior pharyngeal walls and a small pharyngeal residue may result Although the epiglottis may be tilted backward it is not completely turned down The more viscous the bolus the greater the residue. One must distinguish between posture and palsy

I would like to comment on Vernet s curtain movement The clinical sign that saying Ah on stimulation of the pharynx results in an upward and outward movement of the posterior pharyngeal wall has been assumed to be due to unilateral weakness of one of the constrictors. From our limited series we have reason to think that this phenomenon is due to asymmetrical action of the muscles of the soft palate since the patients who exhibit this sign do not always show definite evidence of unilateral weakness of the middle constrictor on swallowin

Dr Bosma seems to us to be unduly influenced by the work of Negus and Pressman who refer to the presence of 3 laryngeal sphincters, and by the assumption that elevation of the larynx and the approximation of the arytenoids to the base of the tongue play a part in its closure We have shown that the laryngeal lumen from the level of the vocal cords upward normally closes from below upward and that this can occur without elevation of the larynx This type of closure may be seen when singing using glottal stop or coup de glotte and may occur when bearing down as in defecation or when the breath is held

In Figure 286 bottom left the open larynx is shown black in this still lateral roentgenogram taken while the patient was quietly breathing Figure 286 bottom right shows the same patie t holding the breath with the larynx closed The lower limits of the vocal cords can be seen clearly or can be in its original form The lumen of the larynx is obliterated from the level upward to the entrance to the larynx There has been no significant degree of elevation of the laryn The upper sections left and right show the same thing taken from a cineradiographic film at 25 frames per second in which the laryngeal structures have been outlined with contrast medium

In the normal subject during swallowin the larynx may be closed at any time during the phase of the descent of the bolus or even before

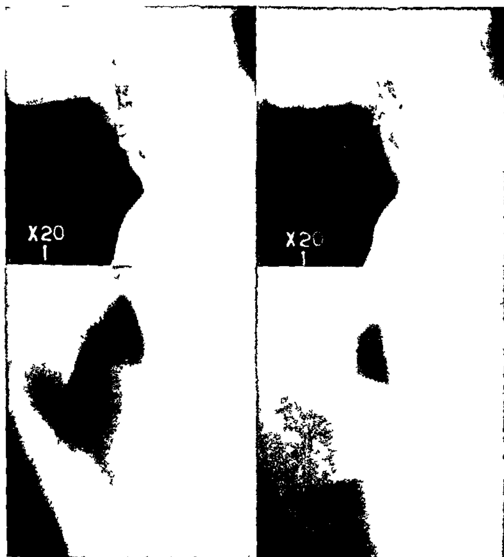


FIG 286 Laryngeal structures

the bolus has left the mouth but it is invariably closed as the bolus is being expressed from the pharynx The laryngeal lumen is usually narrowed but still open as the first of the bolus passes the entrance to the larynx Nonirritant food or contrast medium may enter the vestibule and pass down to but not through the vocal folds and is usually expressed back into the pharynx as the laryngeal lumen is closed from below upward

The 3 upper sections of Figure 287 show the bolus passing through a normal pharynx In the upper left section the epiglottis can be seen outlined against the posterior pharyngeal wall barium spilling on either side of it into the hypopharynx A small amount of barium can be seen in the vallecula and immediately below it a larger barium filled channel shows barium entering the narrowed lumen of the larynx as far as the vocal cords In the original film this could be clearly

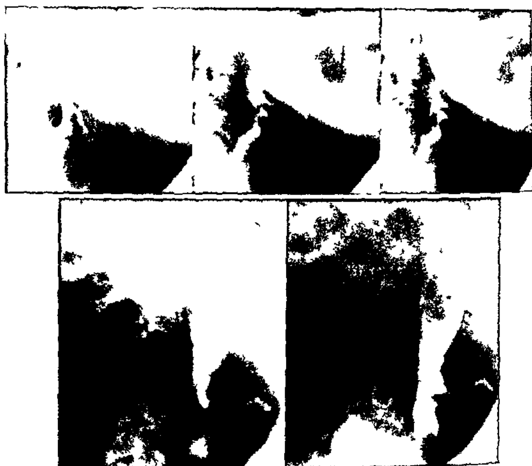


Fig 287 Bolus shown passing through a normal pharynx

seen fanning out in the laryngeal ventricles It is normal for the laryngeal vestibule to be open at this stage In the upper center down by the left there has been further closure of the larynx and the contents of the laryngeal vestibule are being expressed upward into the pharynx The upper right shows a later stage with the vestibule virtually empty The patient was unaware that anything had entered the airway when this occurs it is quite normal for the laryngeal contents to be expressed into the pharynx as the bolus is stripped from the pharynx We would regard the presence of contrast medium in the vestibule as abnormal only when it was not stripped from the laryngeal lumen as the bolus left the pharynx Figure 287 bottom right is a film from another individual showing how contrast medium has passed the entrance to the larynx while the lumen is still open This is the more usual state of affairs rather than that shown in the left hand side

Those individuals who have trained themselves to down a pint of beer and who close the larynx while it is depressed do not show any abnormal laryngeal sphincteric movement they merely have trained themselves to allow passage of fluid through the pharynx while the larynx as a whole is depressed

The most important residual defect in swallowing following poliomyelitis is the inability to clear food from the pharynx This may be d to several causes but in our experience th commonest cause is failure of the pharyn eal constrictor peristaltic wave this may be associated with a failure to move the anterior structures (tongue hyoid and larynx) sufficiently far backward Although elevation and backward tilting of the larynx are not essential for its

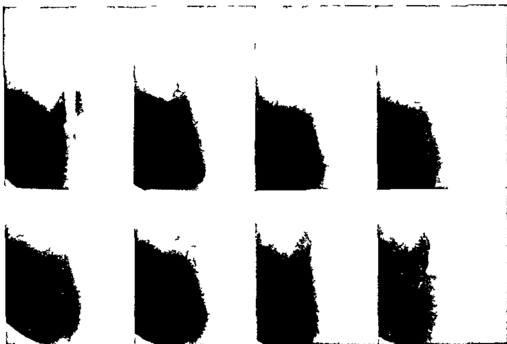


Fig 288 Selected series of frames from a patient with pharyngeal palsy following poliomyelitis

closure they are important particularly in patients with a pharyngeal residue for two reasons If the larynx is not adequately tilted backward there is a much greater tendency for food to enter the lumen If the larynx is not adequately elevated this indicates a failure to shorten the pharynx in a vertical direction which leaves a much greater cavity for the retention of residue That this is so has been seen in at least one patient who had attempted to commit suicide by cutting his throat The wound had severed the pharynx between the hyoid and the larynx and had divided the constrictors at this level Following surgical repair he was found to be quite capable of closing the lumen of his larynx and the pharyngeal constrictor wave passed downward with only a brief break but there was complete inability to elevate the larynx this resulted in a large pharyngeal residue some of which entered the airway on re-opening the larynx We have no experience of operations designed to assist elevation of the larynx in patients with pharyngeal palsy

We have seen some individuals whose laryngeal sphincter appears to be capable of closing quite well but which closes too late after some contrast medium has entered the trachea In our experience the commonest cause of material entering the airway has been spill from the pharyngeal residue as the airway is re-opening

We also have observed what Dr Bosma has described as a failure of opening of the mouth of the esophagus It is not quite clear just which muscle group is involved but we believe it is one of the bundles of the cricopharyngeus

The cricopharyngeal bar referred to by Dr Bosma has been seen by us on many occasions and we are sure that Dr Bosma would have seen it much more frequently had he had the advantage of cineradiography at 25 or more frames per second The contraction producing this sometimes appears before and sometimes after one would expect the peristaltic wave to have reached this region We have seen patients

in whom dilatation of the cricopharyngeus has been of some help or even an operation in which the fibers are partly divided as in Ramstead's operation on the pylorus We would regard cineradiography as an essential method to determine the precise nature and site of the defect before planning any operation

Figure 288 shows a selected series of frames from a patient with pharyngeal palsy following poliomyelitis In the first frame we see the lower pharynx following the swallowing of a mouthful of fluid contrast medium There is a residue remaining In the top left hand corner one can see the outline of the lower jaw and immediately to the right the rather dim outline of the cervical spine in front the hyoid bone and almost immediately below it the black shadow indicating the laryngeal airway Barium residue can be seen coating the walls of the valleculae and lateral food channels and outlining the pyriform fossae and the pharynx immediately above the entrance of the esophagus The next frame shows the larynx elevated and the next bolus descending The laryngeal airway is still wide open The third frame shows that the larynx is now closed The next two frames show the bolus descending and being divided at the level of the oblique fibers of the inferior constrictor The last frame shows the larynx refilling with air A large residue remains in the pharynx the bulk of which will be swallowed by subsequent effort No contrast medium enters the larynx There has been no evidence of pharyngeal constrictor peristalsis gravity and backward movement of the tongue and the larynx have been used Later the forward movement of the tongue and the larynx have contributed to increasing the diameter of the pharynx allowing contrast medium to coat the walls It will be seen that those patients who are unable to alter the size of the pharynx by maneuvers of the tongue and the larynx in the absence of constrictor peristalsis are placed in a most unfavorable position in dealing with a residue It will be readily appreciated that residue might enter the airway on reinflation

BIBLIOGRAPHY

Ardran G M and Kemp F H Closure and opening of the larynx during swallowing Brit *J Radiol 29 205 1956*

——— The mechanism of swallowing Proc Roy Soc Med 44 1038 1951

——— The protection of the laryngeal airway during swallowing Brit J Radiol 25 406 1952

——— A radiographic study of movements of the tongue in swallowing Dent Practitioner 8 252 1955

——— Radiological investigation of pharyngeal and laryngeal palsy Acta radiol 46 446 1956

——— Swallowing without elevation of the larynx J Physiol 126 23 1954

Ardran G M Kemp F H and Manen L Closure of the larynx J Physiol 118 39 1952

——— Closure of the larynx Brit J Radiol 26 497 1953

Ardran G M Kemp F H and Marland P M Laryngeal palsy Brit J Radiol 27 201 1954

Ardran G M Kemp F H and Truelove S C A radiological study of the movements of the tongue pharynx and oesophagus in acrosclerosis Gastroenterologia 79 361 1953

Ardran G M Kemp F H and Wegelius C Swallowing defects after poliomyelitis Brit J Radiol 30 169 1957

Ardran G M and Wyatt D G A portable x ray cinecamera Brit J Radiol 30 557 1957

Bosma J F Studies of disability of the pharynx resultant from poliomyelitis Ann Otol Rhin & Laryng 42 529 1953

Fulford G E Ardran G M and Kemp F H Aglossia congenita Arch Dis Childhood 31 400 1956

Kaplan S Paralysis of deglutition a postmyelitis complication treated by section of the cricopharyngeus muscle Ann Surg 133 572 1951

Kemp F H The movements of the epiglottis during deglutition J Physiol 112 11 1951

Lueck W Galligan J and Bosma J F Persistent sequelae of bulbar poliomyelitis J Pediat 41 549 1952

Naffziger H C Davis C and Bell H G Paralysis of deglutition—surgical correction Ann Surg 128 732 1948

Negus V E The Comparative Anatomy and Physiology of the Larynx New York Grune 1949

Pressman J J and Keleman C P Physiology of the larynx Physiol Rev 35 506 1955

Glossopharyngeal Breathing Medical Management

DR CLARENCE W DAIL

Our first acquaintance with what we later called glossopharyngeal breathing was in 1948 in a young man who had had poliomyelitis 4 years previously We observed that each breath was associated with a strong pull of the neck accessory muscles which was immediately followed by one or more mouth and throat movements that appeared to be gulps of air We found that the patient s vital capacity using his ordinary muscles of breathing was 150 ml but with the aid of these additional gulps of air he could raise his capacity to 600 ml Soon after this experience we observed that several other patients had learned a similar method of breathing independently some by watching other patients We recognized the basic principle of this method of breathing and since it consisted essentially of the use of the tongue the throat and the laryngeal musculature we called it glossopharyngeal breathing We saw a similarity between this method of breathing and that employed by amphibia and therefore also called it frog breathing

The first of our patients to have learned glossopharyngeal breathing as an aid in respiratory muscle paralysis was a woman who had been paralyzed almost completely by poliomyelitis in 1933 She was first seen in our hospital in 1953 and it was observed that she used glossopharyngeal breathing in combination with neck accessory breathing Her ordinary vital capacity was 200 ml but with glossopharyngeal breathing her capacity was 800 ml At that time the patient recounted that she had used this same type of breathing about a year after the onset of her disease that is in 1934 when attempting to free herself from the respirator

The first normal person of whom we have any knowledge of having used glossopharyngeal breathing recalls that at about the age of 6 (in 1934) he used it as an aid in filling his lungs with air before diving He is qualified to make this observation because in 1951 he contracted poliomyelitis and became a respirator patient When we began to teach glossopharyngeal breathing to this patient he told us of his previous knowledge of the method

Since glossopharyngeal breathing has been used more extensively for specific aid in respiratory paralysis occasional reports have reached us of a patient here and there who has learned it without instruction and even without knowing that anyone else had ever used it With our own patients we observed this in 8 cases One could probably conclude that a certain small percentage of all patients who have marked difficulty in breathing will learn it on their own initiative On the basis of this we might further assume that glossopharyngeal breathing probably has been inadvertently used for centuries

Our first report of this breathing was published in 1951 It appears the next report was published in 1954 by Kirschsieper who made an independent report of a patient who developed it following poliomyelitis Kirschsieper compared the breathing of this patient with that of amphibia just as we had done

MECHANISM

We have studied the mechanism of glossopharyngeal breathing with the aid of cineradiography recording the pressure flow rate and volume changes as well as by visual observation of those who have learned it either normal subjects or patients

The mechanism of glossopharyngeal breathing is essentially the same as that of a pump the tongue and the throat structures acting as the piston and the mouth the soft palate and the larynx acting as valves There results a stroke or steplike expansion of the lungs as air is pumped into them When inspiration is completed the inspired air is permitted to escape The procedure has been described elsewhere in greater detail

PHYSIOLOGIC FINDINGS

We have made studies of the blood and the alveolar carbon dioxide tension the arterial oxy

gen tension and arterial pH and have found that even after prolonged glossopharyngeal breathing these values are within normal limits Thus the clinical observation that lung ventilation can be adequately maintained was confirmed by laboratory studies

Glossopharyngeal breathing is an example of positive pressure breathing and consequently may be considered to impair cardiac function For this reason intra arterial blood tension tracings were made during both quiet and deep glossopharyngeal breathing Quiet breathing as used for maintaining ventilation did not produce an appreciable effect on blood pressure but some patients were able to produce a definite drop in arterial tension on expanding the lungs with a deep breath This is borne out clinically Deep glossopharyngeal breathing can be done without producing such effects and still greatly improve the effectiveness of coughing especially when manual assistance is also employed Expiratory air flow rate studies done by us and by Feigelson confirm these observations

CLINICAL OBSERVATIONS

At present the number of our cases doing glossopharyngeal breathing has grown to 272 Of this number 8 learned the technic without any instructions or without any knowledge of the procedure 37 might be considered as having taught themselves after a certain amount of observation such as seeing another patient doing it on the ward The remainder 227 were specifically taught the method 120 of this number were taught by 1 instructor Of the 272 patients 54 could not breathe except by the glossopharyngeal method but with it could breathe an average of 4 hours and 57 minutes with a minimum of 1 hour

Another group of 17 patients had an average breathing ability of 16 minutes Most of the patients in this group were either taught early in our experience and had no follow up endurance training or were very recent cases who were still building endurance Some had extremely weak glossopharyngeal musculature

There was a group of 54 patients who made use of glossopharyngeal breathing as a supplement Finally the largest number of patients (147) were taught the technic only for coughing and chest stretching

CASE PRESENTATION

The patient to be described illustrates some of the advantages indications and management problems in glossopharyngeal breathing

Four years ago at the age of 31 this mother of a 2 year-old child contracted poliomyelitis She was admitted to our hospital 1 month later completely paralyzed except for the muscles of the eyes the face speech and deglutition She had had a tracheotomy and received full-time artificial respiration with a tank respirator The vital capacity was zero

About 4 months after onset glossopharyngeal breathing instruction was started 3 months later the patient had learned the technic but partly because of muscle weakness and partly because of a lack of a good follow up teaching program, it took another 9 months for her to build up her tolerance to 2 hours At that time she had a glossopharyngeal capacity of 1 1/5 ml. in comparison with a regular vital capacity of 225 ml

About 1 month later she could breathe over 7 hours in one stretch but this was not easily maintained since mastication of food at meal times is not compatible with simultaneous glossopharyngeal breathing There is also danger from choking The usual breathing limit per session is 3 to 4 hours Therefore this patient was put on a schedule of 3½ hours twice a day with other shorter periods for care as needed

About 1½ years after onset of the disease the tracheotomy was repaired since she could now cough up her own mucus adequately Incidentally no breathing time was permitted for several days after the repair

The patient s vital capacity now is 260 ml and the glossopharyngeal capacity is now 1 650 ml The improvement in coughing is demonstrated by an expulsive force measurement of only 7 cm water pressure without a deep glossopharyngeal breath and 41 cm with it Her breathing is almost entirely by this acquired means although occasionally she does find it convenient to supplement with the neck accessory muscles.

About 1 year ago the patient was discharged to her home where she has a full time live in attendant She uses a cuirass respirator for sleeping and a rocking bed for rest and feeding She is taken for automobile rides as she

desires She is pushed about in her wheel chair as occasion demands as for example for her weekly visits to the food market She is a regular attendant at church and parent teacher association meetings All this she had done thoughtlessly without any emergency resuscitation equipment along When we found this out only 3 months ago we convinced her that our safety code requires that she always take respiratory equipment with her

The patient lives a happy life with her child who is now 6 and going to school She reads books considerably and is able to turn the pages with the aid of a properly designed book rack and a mouth stick She does oil painting also with the aid of mouth and neck movements If she desires she also can operate a typewriter by this means

TEACHING GLOSSOPHARYNGEAL BREATHING

In our experience glossopharyngeal breathing is best learned by a definite program of individual instruction Our first patients had to learn by observation of others who had already mastered the techni They also received occasional encouragement from the hospital personnel Before we organized an effective teaching program 8 to 10 patients learned glossopharyngeal breathing each year Aft r we initiated the program the number rose to 55 then to 85 The teacher himself should know how to do glossopharyngeal breathing so that he can speak from experience and can demonstrate the steps in the technic

A manual of instruction has been published This is based on several years of experience in teaching and now about 3 years after preparation it still represents our basic method

Glossopharyngeal breathing is obviously indicated as a substitute method when the patient has no expected functional return of normal breathing but still possesses strength in the muscles of the mouth the throat and the larynx sufficient to perform th act It is also indicated when the ordinary breathing muscles need assistance or rest

Since glossopharyngeal movements can be employed to expand the lungs fully they are useful in improving a weak cough Furthermore we believe that it is a valuable method of lung and chest stretching in all conditions that cause a decrease in lung and chest flexibility or compliance Although there is not positive proof of this clinical observations do indicate its value When the patient expands his lungs sufficiently to cough or stret h there may develop enough intrapleural pressure to produce the Valsalva effect It is our practice to instruct the patient how much to expand his lungs after checking the effect on the pulse and after noting any possible symptoms of fullness in the head dizziness or other circulatory symptoms Usually this is no problem and so far we have observed no evidence of injury

It is important that the patient be enthusiasti about the program Therefore he should be well oriented and fully informed about the advantages of learning He should not entertain the thought that hope is given up for the return of normal breathing just because glossopharyngeal breathing is now being started Actually it is an aid in the return of expected normal breathing

A quiet environment should be furnished so that the patient can concentrate There must be no situation which may cause emotional disturbance The patient should possess confidence in learning but he should understand that it may take considerable time and effort

If there is weakness of the muscles which are used the patient should be taught exercises which will strengthen them as well as build up endurance before instruction of actual glossopharyngeal breathing begins The lessons should not be too long 15 minutes seems to be the ordinary optimum time Daily instruction is desirable

The program for bodily activity or any other program which may tire the patient may have to be limited during the training period This may include such procedures as freeing the patient from a tank respirator to use a cuirass or (chest) respirator gymnasium activity or surgery

We have found that training a patient who has only a few minutes breathing tolerance requires the proper type of respiratory aid One should be able to stop and start the aid instantaneously For this reason we prefer either the cuirass body type or mouth positive pressure respiratory aid

Evaluation of accomplishment during the course of training is necessary Without a cer

tain knowledge that the technic is effective much time can be wasted furthermore the student may have to learn tediously to correct a bad breathing habit There are various ways of determining effectiveness One should watch the chest and the abdomen to see if they are made to expand passively by small step movements If the patient can cough and call definitely louder with the help of glossopharyngeal breathing this is further evidence of success Improvement of vital capacity is also a good index However it has been our experience that the best index of accomplishment is the spirometric tracing which shows steplike increase *in lung volume A suitable metabolism apparatus* serves as a satisfactory spirometer One must use a comfortable face mask which will not interfere much with the movements

When a patient has learned the technic he may not yet have sufficient strength and tolerance to breathe more than 3 or 4 minutes by this method There must be a continuation of diligence for it may take months before breathing can be sustained for more than an hour as was true in the case presented All cases should be taught how to expand the chest deeply and also how to improve the cough by this method Patients who are taught glossopharyngeal breathing for coughing should not forget to make it a practice to take several strong coughs daily so that the for e is well maintained for the purpose of combatting future respiratory infections

If the patient has a tracheotomy tube he must be able to keep it stoppered for most of the time except when he needs suctioning Excessive tracheal mucus may be a great hindrance to learning the technic of glossopharyngeal breathing If it is still necessary for a tracheotomy tube to be used and the patient s tube is stoppered great care must be taken that the stopper does not pop out The patient with respiratory paralysis thus may have no means of calling the attendant and no means of breathing

SUMMARY

The development mechanism certain clinical aspects principles of teaching and medical management of glossopharyngeal breathing are discussed A case with respiratory paralysis is presented When it is indicated and properly applied we have not observed any detrimental effect In the absence of any appreciable weakness in the participating muscles patients have been able to learn the procedure in nearly every case although it may occasionally take many months Its use has proved to be of gratifying value in many patients with respiratory muscle weakness or paralysis in furnishing independent breathing time as well as improving lung expansion and cough In many patients it may have been shown to be of help in facilitating their *care There may have been several occasions* when its use has appeared to be life saving

BIBLIOGRAPHY

Affeldt J E Dail C W Collier C R and Farr A F Glossopharyngeal breathing ventilation studies J Appl Physiol 8 111 1955

Collier C R, Dail C W and Affeldt J E Mechanics of glossopharyngeal breathing J Appl Physiol 8 580 1956

Dail C W Glossopharyngeal breathing by paralyzed patients, California Med 75 15 1951

Dail C W and Affeldt J E Glossopharyngeal Breathing 16 mm motion picture with sound produced by Dail and Affeldt distributed by the National Foundation for Infantile Paralysis Inc New York

Dail C W Affeldt J E and Collier C R Clinical aspects of glossopharyngeal breathing report of its use by one hundred postpoliomyelitic patients J A M A 158 445 1955

Dail C W Zumwalt M and Adkins H A Manual of Instruction for Glossopharyngeal *Breathing* New York National Foundation for Infantile Paralysis 1955

Feigelson C I Dickinson D G Talner N S and Wilson J L Glossopharyngeal breathing as an aid to the coughing mechanism in the patient with chronic poliomyelitis in a respirator New England J Med 254 611 1956

Kirschsieper H M Eigenartiger Atemhilfsmechanismus bei Peripherer Poliomyelitischer Atemlahmung Arch Kinderheilkunde 148 258 1954

DR. KIRSCHSTEPER. When a few years ago we studied replacement ventilation which was continuously observed in a polio patient (Fig 289) we also had the idea as did Dr Dail that here was a function which had been forgotten completely and could replace normal ventilation Such an assumption might be somewhat romantic at first view because it includes a phylogenetic aspect In this respect we should not forget that genetic history and viewpoints of this history have made a considerable contribution to our concepts on the development and the construction in the human organism The spontaneous occurrence of glossopharyngeal ventilation as a replacement function and as a result of poliomyelitis paralysis of respiration is not the only proof that this auto-function remains within the realm of the human being There are quite a number of observations which tend to prove that glossopharyngeal movements occur in the normal respiration This is not only in agony or in the collapse of respiration where apart from shock respiration there might also be a deglutition particularly in premature children Such movements also are found in narcosis and as a result of any hypoventilation Further divers and swimmers know that such movements can counter the lack of respiration Beyond that however healthy persons can learn glossopharyngeal breathing Before we consider the problems involved let us look into amphibial breathing for comparison from a physiologic point of view

However one cannot expect that the form of respiration is always in line with those of amphibia since in that type of animal there are also differences in the type of application of this respiration Respiration through the skin plays an important part in that they have special epithelium for this

Schematically glossopharyngeal breathing can be subdivided into 4 parts (Fig 290)

1 When the nose is opened There you have an oscillation which leads to renovation of the air in the mouth

2 If you close the nose and open the valve you have the air pushed into the mouth

3 If you raise the palate this air is pumped into the lungs that is inspiration This is under B and C

4 The valve is closed and the nose opened and there is a new ventilation in this way the respiratory mechanism starts all over again

If the animal is excited only pressor lung respiration occurs That is the upper graph as can be seen from the registration of the considerable oscillations (Fig 291) If the animal calms down then the oscillations are seen in the lower graph If the animal is completely quiet these oscillations are overwhelming and the synchronized occurrence of the considerable oscillations can be seen (Fig 292)

A number of oscillations are registered which might be in line with the pump movements and with the respiratory work of our patient with glossopharyngeal breathing The same applies to the supplementary breathing in the human being (Fig 293) when (1) the air comes into the mouth by opening (2) the nose and the mouth are closed and during that period the larynx button is also closed (3) if the larynx is opened and the tongue and the muscles moved then you have them pumping in air into the respiratory circle then we have (4) the larynx closed again

A number of pumping movements in the human being then lead to expiration In the human being we have the same oscillations and pump frequencies which depend on the number of physical incentives and irritations Now in a typical amphibian the pump-pressure respiration is dominant in the reptile the thoracic breathing is dominant Of course this is not a clear-cut separation for instance the agyptum solamentum shows signs where you can study the pump work You never have thoracic breathing and glossopharyngeal breathing at the same time It is only when you come to the higher class of animals that you have the separation clear-cut This also leads to a stronger and stronger impediment to the lower layers which is only reactivated when you eliminate the central state In this way the observations

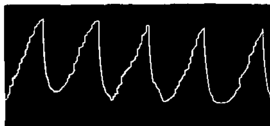


Fig 289 Original respiratory curve (registered by the spirograph) of a 12 year-old child with glossopharyngeal breathing

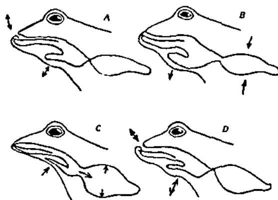


Fig 290 Schematic drawing of pump respiration of a frog (described in text)

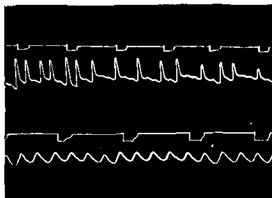


Fig 291 Original curve of the gutteral movement and gutteral oscillation of a frog (*Top*) Aroused animal Each gutteral movement is bound with a pressurized lung ventilation (*Bottom*) Animal at rest The curve records additional gutteral oscillations

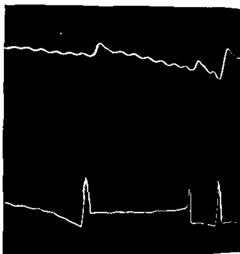


Fig 292 Original curve Synchronous recording of the thorax movement (*top*) and the gutteral movement (*bottom*) in a frog

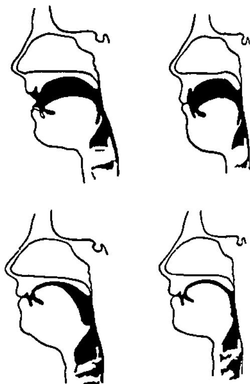


Fig 293 Schematic drawing of pump respiration in a human (described in text)

we have made on the deglutition and glossopharyngeal breathing can be explained However we must not forget that the exact functional organization which leads to the glossopharyngeal varieties cannot be described in detail yet

A few observations lead to the conclusion that it was not only the change of the CO_2—O_2 tension in the blood that played a part but also general exercise can be possible and can lead to glossopharyngeal breathing The breathing mechanism generally occurs spontaneously only a certain period after respiratory paralysis but then can become automatic to such a strong degree that it makes not only eating and drinking possible but also is in line with sleeping Even during sleeping after you stop the tank respirator you can stop it without having the patient notice it

Care of Patients Severely Stricken by Poliomyelitis

WEDNESDAY AFTERNOON, JULY 10, 1957

(This Session Convened in the Aula of the University of Geneva)

Chairman

PROF G FANCONI
University of Zurich
Zurich

Speakers

DR JAMES L WILSON
University of Michigan Medical School
Ann Arbor

DR H C A LASSEN
Blegdamshospitalet
Copenhagen

DR HORACE L HODES
The Mount Sinai Hospital
New York

DR W RITCHIE RUSSELL
United Oxford Hospitals
Oxford

DR BJORN IBSEN
Kommunehospitalet
Copenhagen

DR ROBERT J EIBEN
City Hospital
Cleveland

DR GORAN HAGLUND
Epidemic Hospital
Gothenburg

DR GEORGE SAXTON JR
Poliomyelitis Respiratory Center
Research and Educational Hospital
Chicago

Discussants

DR DAVID C DICKINSON
University of Michigan Medical School
Ann Arbor

PROF DR E ROSSI
Universitats Kinderklinik
Berne

DR BENJAMIN G FERRIS
Department of Physiology
Harvard University
School of Public Health
Boston

Introduction

DR JAMES L WILSON

Only the enormous advances made in preventing poliomyelitis by active immunization can permit some contentment with the lack of progress in developing any form of specific therapy that influences directly or effectively the action of the virus in the central nervous system About all we can do on a reasonably scientific basis during the acute stage of poliomyelitis is to make use of apparatus and technics not to influence the course of paralysis but to save life In this Fourth Congress on Poliomyelitis as in the preceding ones the problems of saving life by mechanical technics have dominated the session on the care of patients in the acute stage of disease The same emphasis will be made in this program but we hope that considerable attention will b given also toward making this saved life a more useful one

To a great extent the problem of saving life with only paralysis of respiratory muscles remaining has been successfully met To a lesser extent but still with considerable success the problem of saving life has been solved in patients who with or without respiratory muscle paralysis have difficulty in breathing because of the paralysis of their pharyngeal muscles Limited progress only has been made in handling patients with acute brain stem disease that may *disturb not only the control and efficiency of* respiration but also the vasomotor system with resulting abnormalities in blood pressure pulmonary edema and other manifestations of shock Studies of these victims at autopsy with appreciation of the vast extent of the damage done by the virus makes us realize that it is improbable that any therapeutic technics can be successful in these patients until an antiviral agent has been found

In any consideration of these lifesaving procedures certain moral problems continuously arise as they did in fact with the first patients with respiratory muscle paralysis who were treated with prolonged artificial respiration The question does arise whether a physician has a duty or even a right to save the life of a patient doomed to permanent and complete helplessness Most physicians do not believe that they should accept the responsibility for euthanasia even by inaction But in any case the clinical course of poliomyelitis shows such great spontaneous variations that a dependable prognosis in the acute stage is impossible Patients who at first seem most disastrously paralyzed do sometimes show remarkable recovery so that it is impossible for a physician who might wish to make a decision in the acute stage of poliomyelitis as to whether his patient s life was worth saving or not, to do so with any certainty

In the past many of us have given altogether too little attention to the after-care of the life which we have saved by artificial respiration during the acute stage of poliomyelitis This has been particularly true in the United States, where many lives have been saved in the acute stage by the efficient use of the widely available tank respirator but such patients have been left dependent upon this device for months or many years or even for the rest of their lives Much progress has been made recently toward success in returning these patients to their homes free of cumbersome mechanical aids to respiration Few patients with respiratory muscle paralysis, saved from death by artificial respiration need to continue permanently dependent upon such apparatus for respiratory assistance Such a patient almost certainly can be freed from handicapping apparatus for most of the hours of the day at least and usually can be returned with care to his home by persons without technical skill or training

But saving a life and thus freeing a patient to a great extent from dependence on artificial aids to respiration does not in fact complete our medical obligation to this tragically crippled person We may all believe that each is born with the right to life and personal liberty but there is another right which perhaps should be

accepted as equal to these one to which the medical profession can give great assistance that is the right to be useful This ultimate in rehabilitation must not be neglected by physicians. How can we make our patients who have been freed from respiratory aids also become useful perhaps to earn a living certainly to take care of their own persons and possibly their homes and to get further training and education? We feel happy that great progress has been made in attaining such ends with extensively paralyzed patients and although at times this has been accomplished only at great cost, it is a cost quickly repaid by a few successes When two thirds of a group of quadriplegic patients who have been confined to respirators for many months have been able with skilled assistance to progress to a point of earning an honest living we can claim important progress even though it may be pathetically little as compared with the prevention of the disease or the hope that sometime we actually may cure it during the acute stage when prevention has not been accomplished

Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances

DR H C A LASSEN

In the acute state of poliomyelitis and during the first few weeks after the most frequent cause of death is respiratory insufficiency with its complications obstruction of the airway atelectasis and pneumonia pulmonary edema and circulatory shock with kidney damage and quite often also signs of impairment of deglutition and symptoms of involvement of the autonomic centers in the brain stem In some few cases death can be ascribed to polioencephalitis with hyperpyrexia

It seems surprising that so relatively little has been published regarding the particulars of the complex clinical pictures of this disease which endangers life and practically nowhere has any attempt been made to correlate death rates with treatment This no doubt is due to the fact that until recently therapeutic results in the severe forms were uniformly disappointing if not downright discouraging

However this gloomy picture seems to have changed somewhat for the better in recent years But still we are far from sure that our improved understanding of the underlying pathophysiologic conditions and our more intelligent interpretation of biochemical effects—which have given rise to better therapeutic equipment—can be made responsible for this amelioration of prognosis In fact this is to be doubted

An immense number of data on death rates and on the incidence of the different clinical forms of poliomyelitis are on record In many countries notification has been obligatory for decades Most often death rates are computed on the basis of the total number of cases notified which of course is misleading Fatality rates should be based only on paralytic cases because first death occurs only in patients with paralysis and second while the diagnosis of paralytic poliomyelitis can be assumed to be fairly correct—especially in epidemic times—the diagnosis of nonparalytic meningitic poliomyelitis not to mention the milder forms is often impossible to make with any certainty It follows that fatality rates based on such data can never be even reasonably correct Only the health authorities in Sweden the United Kingdom and Denmark distinguish between paralytic and nonparalytic cases

Besides the importance of correct diagnosis and conscientious notification other factors influencing statistics should be kept in mind before a fair comparison of data from different countries and at different times is attempted

Fatality rates are usually higher in endemic than in epidemic cases Males generally have higher death rates than females especially in the older age groups and as a whole mortality increases with increasing age in both sexes Finally poliomyelitis like infectious diseases in general is never static The epidemiologic pattern is steadily changing from the primitive type in which clinical disease is quite rare and mostly appears in small children toward the type now prevailing in countries with a long experience of poliomyelitis in which the disease seems constantly to move upward in the age groups

From clinical experience we know that the severity of epidemics varies considerably Even virus strains belonging to the same type may show great differences of virulence in laboratory experiments and this no doubt must play a role in the epidemicity and the severity of the disease However this feature is difficult to assess and can be described only objectively by stating the incidence of life threatening complications and their relation to mortality

In Table 157 the relation between mortality and certain complications is evident Some of these complications especially hyperpyrexia and probably edema carry high mortality rates These figures are from the epidemic in Copenhagen in 1952 where we had 1,235 paralytic cases including 333 with respiratory insufficiency with or without impairment of deglutition

TABLE 157 COMPLICATIONS INCIDENCE AND RELATION TO MORTALITY (345 CASES—MORTALITY 4? PER CENT)

COMPLICATIONS	INCIDENCE		MORTALITY	
	NO OF CASES	PER CENT	NO OF CASES	PER CENT
Shock	134	39	90	67
Hyperpyrexia	66	19	60	91
Uremia	36	22	28	78
Pulmonary edema	29	8	26	93
Paralytic ileus	112	32	49	44
Hypertension	65	19	27	42

Only p n nts f age 15 r n or

These features all combine when characterizing epidemics and are of decisive importance to *mortality In other words spontaneous* differen es are so great that critical evaluation of one or another type of treatment in respect to mortality is extremely difficult if not impossible at least if the life-theatening ases are not a curately described and carefully grouped

We need an intelligent classification and we ought to try to find one on which we all can agree Such a classification should be simple unequivocal and sufficiently detai ed on all decisive points We should state our diagnostic criteria and our therapeutic indications clearly The usual anatomic classification obviously is not satisfactory

As an unobstructed airway prognostically is of the greatest importance in cases with reduced alveolar ventilation the simplest way of grouping these patients is to divide them into two categories dry and wet Wet is here defined as accumulation of secretions in the lower respiratory pa sages Therapeutic indications are different in these two categories and the prognosis is aggravated significantly in the wet group

Apart from diagnostic criteria which we ought to find a way of standardizing an intelligent description of therapeutic indications is equally important before we attempt to compare the effect of different therapeutic measures su h as indications for doing a tracheotomy for the use of tank respirators or for the use of an intratracheal positive pressure machine If tank respirators are used on wide and not specifically stated indications on patients solely designated as respirator patients results naturally will be comparatively favorable

Yet even when most of the points hitherto mentioned are tal en into account comparison of series of patients from different clinics is still extremely difficult probably becaus we cannot describe adequately the severity of the disease This is well illustrated by comparing fatality rates in the Copenhagen epidemic in 1952 with the figures published by our Swedish colleagues in their brilliant study of the Stockholm epidemic in 1953 Their diagnostic criteria and therapeutic indications were very much

TABLE 158 DEATH RATES OF PATIENTS CORRELATED WITH TYPES OF CASES

	COPENHAGEN 195		STOCKHOLM 1953	
	NUMBER	FATALITY RATE PER CENT	NUMBER	FATALITY RATE PER CENT
Deaths	102		27	
Paralytic cases	1130	9 0	633	4 3
Respiratory in ufficiency	205	48 3	144	18 8
Respirator tr atment	154	54 5	89	30 3

TABLE 159 EVOLUTION TIME OF RESPIRATORY INSUFFICIENCY IN 333 PATIENTS

EVOLUTION TIME	NO OF PATIENTS	DEATHS	MORTALITY RATE PER CENT
Less than 2 days	160	85	53
More than 2 days	149	50	34
Not established	24	6	(?5)
Total	333	141	4?

like ours and the epidemiologic pattern of the disease is nearly identical in the two countries

In Table 158 death rates are correlated with the number of paralytic cases the number of patients with respiratory insufficiency and the number of patients who had respirator treatment Fatality rates in all groups were highest in the Danish epidemic although we had practically the same indications in the two countries This to some extent must be due to a greater severity in the Copenhagen epidemic even if such a difference is not proven Yet it is possible to assess the effect of the therapy introduced by us in 1952 by comparing the fatality rates in our hospital before and after the new therapeutic measures were introduced Before this the fatality rate was over 80 per cent at the end of the epidemic it had fallen to 25 per cent despite seemingly unabating severity of the respiratory cases

The time of evolution of respiratory insufficiency has some bearing on prognosis. Table 159 shows that when respiratory failure develops rapidly the prognosis is less favorable than in cases with a more protracted evolution time The duration of artificial ventilation—here called ventilation time—also is related to prognosis

From Figure 294 it will be observed that in about 30 per cent (56 out of 194) of the cases artificial ventilation was not necessary As to the remaining 138 patients, the curve indicates that within the first 2 or 3 months of artificial ventilation the individual patient still has a good chance of regaining adequate spontaneous respiration After 6 to 9 months the prospects of regaining full spontaneous respiration are only slight Thus of the 102 patients who after 1 month were still in respirators no less than 43 had to be given continuous artificial ventilation including 25 who eventually became chronic

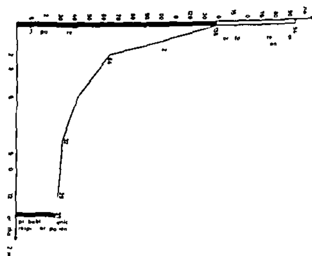


FIG 294 Chart of patients with respiratory failure showing number of those requiring artificial respiration number not requiring artificial respiration and number of chronic respirator patients

respirator patients These patients all had severe paralysis of the diaphragm and the intercostal muscles However it is worth stressing that the diaphragm may start regaining contractility very late In some cases it may take 5 to 6 months before active contractions commence but even then normal function can be attained

Our 25 chronic respirator patients have been ventilated continuously by means of intra tracheal positive pressure machines and 18 are still in the hospital nearly 5 years after the acute stage Three have died 2 in hospital 1 quite suddenly from cardiac failure and 1 from calcu lous pyelonephritis but none from pulmonary complications The majority have renal calculi and intractable infection while stone formation is quite rare even in severely paralyzed patients who have not had artificial ventilation A few have been operated on but in most cases the stones constantly grow although in some few instances they have been observed to disappear spontaneously Probably most of these unfortu nates eventually will die from uremia

Since vaccination was started in Denmark in 1955 on a wide scale—98 per cent of the chil dren and more than half of all adults have now had at least two injections—we have had little poliomyelitis and only 1 respirator case in an unvaccinated girl Whether this is due to the vaccination I do not know Yet from other evidence I feel confident that—now at long last—thanks first to the brilliant work of our Ameri can colleagues we can see the end of respiratory insufficiency in poliomyelitis

BIBLIOGRAPHY

Bower A G Bennett, V R Dillon J B and Axelson B Ann West Med & Surg 4 561 1950

Freyche M and Nielsen J World Health Or ganization Monograph Series No 26 Geneva 1955

Hamtoft H Ugesk laeger 115 1226 1953

Lassen H C A Management of Life Threat ening Poliomyelitis Edinburgh Livingstone 1956

——— Med Press 232 443 1954

Neukirch F Helvet paediat acta 9 381 1954

Søttrup T *In* Lassen H C A Management of Life Threatening Poliomyelitis p 94 Edin burgh Livingstone 1956

Strom J The poliomyelitis epidemic in Stock holm 1953 Acta med scandinav Supp 316 1956

Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances

DR HORACE L HODES

I will discuss in part along the lines which Dr Lassen started A discussion of statistics regarding the mortality and prognosis of patients with respiratory disturbances must take into account the fa t that poliomyelitis may bring about respiratory difficulty in a number of different ways These difficulties we list generally as four

1 That caused by injury of the nerve cells in the medulla which control respiration

2 That due to obstruction of the respiratory tract which is caused by weakness of the muscles of swallowing

3 That due to impairment of functions of the respiratory muscles themselves

4 Finally common and most important various combinations of all of these first three types

Prognosis is different in each of these categories both for survival and for ultimate freedom from mechanical respiratory devices For a number of reasons prognosis for immediate survival is poorest among patients with a central respiratory disorder For example the central control of systemic and pulmonary blood pressure is often severely disturbed in these patients so that death from shock or from pulmonary edema may occur even when adequate pulmonary ventilation can be furnished to the patient by mechanical means

With modern methods of treatment survival is the almost universal rule when respiratory difficulty is caused by obstruction of the airway due to weakness of the muscles of swallowing that is provided that this is the only cause of respiratory difficulty As for the muscles of respiration themselves a rough index of the prognosis for patients with this disability can be obtained by serial measurements of the vital capacity For example a survival rate of about 100 per cent may be expected among patients whose vital capacity in the acute phase of uncomplicated respiratory muscle poliomyelitis does not fall below 75 per cent of the expected normal value Furthermore such patients rarely need mechanical respiratory apparatus Patients whose vital capacity does not fall below 50 per cent are in a similar position provided that their breathing difficulty is due entirely to respiratory muscle weakness

When the vital capacity does fall below 35 per cent of normal the patient may be in danger of dying unless artificial respiration is provided In attempting to give a prognosis in a specific case we must realize that the vital capacity is only a rough guide to prognosis and that many other factors are involved For example a patient may be able to meet his respiratory needs with a vital capacity of 30 per cent if this ventilation is achieved entirely by motion of the diaphragm In contrast a patient with the same vital capacity which is achieved by contraction of accessory muscles of the neck may be able to breathe unassisted for only a few hours Prognosis then is different in each of the two cases

During the past 10 years improved medical care has reduced the case fatality rate greatly among poliomyelitis patients with respiratory difficulties The chance of attaining freedom from mechanical devices also has been improved greatly as Dr Wilson said These changes are due to a number of factors and improvements

The progrosis for poliomyelitis patients with respiratory impairment has improved in the last 10 years The question is can we give a more a curate estimate of the prognosis than this encouraging generalization? An accurate estimate is beset with difficulties some of which I shall consider briefly

First the outlook for a given series of patients will depend to a large degree on the number of patients with central respiratory disease in the group The result attained with a series which includes a high percentage of such cases

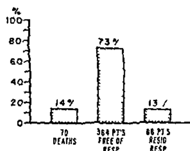


Fig 295 Two year observation of 500 consecutive respirator patients indicating mortality respirator freedom and residual respirator incidence (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

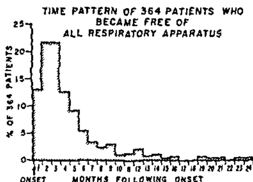


Fig 296 Three hundred sixty four of the 500 respirator patients became free of all respiratory equipment within 2 years of onset The time pattern for this is presented by 6 months 83 per cent of the 364 had become free (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

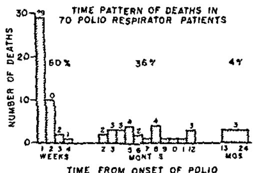


Fig 297 Seventy out of the 500 patients (14%) died within 2 years of disease onset Over 50 per cent of the fatalities occurred during the first 2 weeks (Affeldt J E *et al* Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

will be much worse than that with a group made up almost *entirely of patients with respiratory muscle weakness* A second important variable concerns the difference in the criteria used for deciding whether or not respiratory involvement is present For example one investigator may search carefully for minor degrees of respiratory muscle impairment and he then may include patients with minimal respiratory loss in his statistical study A second physician may fail to detect slight respiratory muscle impairment or he may exclude such patients from consideration deliberately on the grounds that they are not in danger The over all prognosis will appear to be much better for the patients of the first investigator than for patients in the second group Care must be taken to avoid attributing such apparent difference to a particular form of treatment or indeed to a particular special type of apparatus Finally the skill and experience of the physicians nurses and other attendants have a direct bearing upon prognosis and a team meeting its first epidemic of poliomyelitis will not do as well as will one with previous experience

Now the variable factors which we have discussed would tend to be cancelled out in a large series of patients A study of such a group carried out with criteria as uniform as possible should yield an accurate estimate of over-all prognosis Such studies have indeed been carried out in a number of places One was carried out by Dr Affeldt and his colleagues in Los Angeles and one by Spencer and Jackson in Houston.

Affeldt s study is concerned with patients with severe respiratory difficulty and it can be assumed that the results which he reports are less favorable than those which we might expect

with less severely affected patients Affeldt studied 500 consecutive polio patients who were placed in tank respirators in the Los Angeles County Hospital the study running between January 1 1950 and August 15 1953

At the Los Angeles County Hospital the vital capacity is routinely measured for acute polio patients A poliomyelitis patient is placed in a tank respirator if his vital capacity falls to approximately 30 per cent of the predicted normal This means that nearly all of the 500 patients in Affeldt s study had a vital capacity of 30 per cent or less of the predicted normal on admission to the hospital or soon after

Affeldt s patients were observed for a minimum of 2 years and his over all results are shown in Figure 295 Of the 500 patients listed 70 died a case fatality rate of 14 per cent

Sixty six or 13 per cent of the patients were still using respiratory equipment 2 years after the onset of poliomyelitis Some of these 66 patients required the respirator some a rocking bed and others only a cuirass respirator Of the 66 patients still using mechanical devices 27 employed these only at night These 66 patients included those who used the respirator part or all of the day We see that 364 of Affeldt s patients 73 per cent became completely free of all mechanical respiratory equipment Freedom from respiratory devices was attained most often during the second and third month after onset of illness Eighty three per cent of those who eventually became free of mechanical devices had done so by the time 6 months had elapsed However 1 or 2 patients each month achieved freedom up to 2 years after onset This is shown in Figure 296

Well we see that a vast majority of patients became free of respiratory difficulties by about the sixth month There is a scattered improvement up until the period of 2 years After that time freedom from respiratory equipment of all kinds is much less likely to occur

Among Affeldt s patients death occurred most frequently during the first week of disease About half of the 70 fatalities were experienced during this period Of all the deaths which took place during the 2 year study 60 per cent occurred during the first 4 weeks of illness Thirty six per cent of the deaths occurred from the second to the twelfth month and 4 per cent

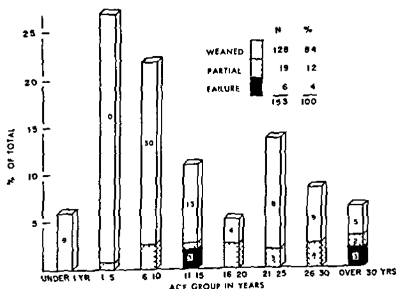


FIG 298 Respiratory outcome of 153 acute poliomyelitis respirator patients requiring more than 2 weeks of respiratory assistance (Spencer W A and Jackson R R Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans 1956)

occurred between the thirteenth and twenty fourth month as shown in Figure 297

Affeldt's results are similar to those obtained by Spencer and Jackson although the data were arranged in a somewhat different manner in the two studies

Spencer and Jackson's data (Fig 298) deal only with patients who required tank respirator treatment for a period of 2 weeks or longer Furthermore patients who needed respiratory aid for less than 2 weeks were excluded and also those who died within 2 weeks after contracting poliomyelitis Altogether these authors studied 153 respirator patients who were treated by them from the onset of the illness The patients in the group were observed from 6 to 76 months with the great majority being studied over 1 year

Of the 153 patients 128 (84 per cent) became entirely independent of all respiratory devices Nineteen patients (12 per cent) were partially weaned requiring mechanical assistance of some kind for more than 30 minutes but less than 24 hours a day Six patients (4 per cent) failed to become free of mechanical assistance for more than 30 minutes a day

CONCLUSION

1 Under favorable hospital conditions we may expect an over all 2 year case fatality rate approximating 15 per cent Eighty five per cent may be expected to survive for a period of more than 2 years

2 Approximately 85 per cent of those who do survive the acute phase will become independent of all respiratory apparatus within 2 years The great majority will achieve this goal within 6 months after the onset of illness

3 At the end of 2 years approximately 15 per cent of the survivors will still require some form of mechanical respiratory assistance for some portion of each 24 hour period Very little improvement can be expected beyond the 2 year period In fact a mortality rate beyond that of normal persons can be expected in these patients Regarding permanent use of respiratory equipment it is known to the National Foundation for Infantile Paralysis that there are in the United States 1 700 patients who use mechanical respiratory aids for at least part of each day

4 Finally what is the outlook for poliomyelitis patients with minimal and mild loss of respiratory function? Clearly this must be much better than the statistics which I have just quoted Exact data on this point are not available

BIBLIOGRAPHY

Affeldt J E Bower A G Dail C W and Nobby N A Prognosis for respiratory recovery Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans Nov 25 1956

Blossom R A and Affeldt J E Chronic poliomyelitic respirator deaths Am J Med 20 77 1956

Hodes H L Treatment of respiratory difficulty in poliomyelitis *in* Poliomyelitis Papers and Discussions Presented at the Third International Poliomyelitis Conference Philadelphia Lippincott 1955

Marchand J F and Marcu A T Respiratory recovery rates after poliomyelitis Am J Med 17 683 1954

Spencer W A and Jackson R R Respiratory outcome of 153 acute poliomyelitis respirator patients requiring more than 2 weeks of respiratory assistance and respiratory outcome among 167 chronic respirator patients Paper presented at meeting of Respirator Center Committee of National Foundation for Infantile Paralysis New Orleans Nov 25 1956

Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances

DR W RITCHIE RUSSELL

Twenty years ago the case fatality rate in some large outbreaks of poliomyelitis was more than 20 per cent of all paralytic cases and clinicians will agree that the cause of death in the great majority of the cases listed must have been some form of respiratory insufficiency

The methods of treatment developed in the past 20 years have changed this picture completely so that the paralytic case fatality rate should now in general be below 5 per 100 paralytic cases and in some large outbreaks has been brought down to a much lower figure Thus I think it should be mentioned that in Los Angeles County California in 1954 to 1955 there were over 1 000 paralytic cases of which 175 required respiratory aids or tracheotomy and yet *only 15 died—a paralytic case fatality rate* of less than 1 4 per cent This is surely a record and one to be proud of!

This improvement in mortality began first in the United States in relation to the interest forced on the medical profession by the great epidemics in the 1930 s In some respects the methods developed in the United States for saving life in acute poliomyelitis have until recently remained far ahead of those used in other countries of the world thanks partly to the remarkable assistance in research provided by the National Foundation and partly to the disorganization of research in Europe brought about by the second great war

To some extent statistics now can be used to study these changes owing to the surprising fact *that during the past 10 years in both the United* States and Britain the case fatality rate seems to have been almost entirely uninfluenced by the type and severity of the epidemics that have occurred

Twenty years ago the case fatality rate varied considerably from year to year but it seems that modern methods of saving life have abolished this variation to a large extent as there is no evidence to suggest that the disease has become less severe On the contrary these figures suggest that this proportion of life threatening cases remains fairly constant from one outbreak to another and as in the great epidemic in Denmark of 1952 one quarter to one third of all paralytic cases are life threatening in large outbreaks of the disease—I think perhaps in all countries of the world I think the idea that the stability of the outbreaks changes much from one outbreak to another is countered by the statistics of the last 10 years

The steadily declining level in case fatality rates is simply an indication of improved methods of saving life during the acute stage If this is so then we may conclude that the best and easiest indication of *the standard of treatment* in any one outbreak is provided simply by the case fatality rate for the paralytic cases in that outbreak

Only in recent years have suitable statistics become available for such studies and indeed the figures for England and Wales are the only ones that I have seen in which there is both an adequate separation of paralytic from nonparalytic cases and at the same time sufficient cases in each year for analysis and comparison of one year with the next The Danish reports separate paralytic cases but in many years they have listed few patients Some of the United States reports separate paralytic cases from nonparalytic ones but there is a large unspecified category so that for the whole country the paralytic *case fatality rate can be assessed only approximately* by assuming that the proportion of paralytic to nonparalytic cases in designated cases remains the same in those which are reported as unspecified This calculation has been incorporated in Table 160 and the figures from which this is prepared are published in the *Excerpta Medica* with which we all have been provided

TABLE 160 NOTIFICATIONS AND CASE FATALITY RATES FROM 1945 TO 1956 FOR ENGLAND AND WALES (E & W) AND UNITED STATES (U S A)

Poliomyelitis		1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Total notifications per 100 000	E & W	2 0	2 6	18 1	4 3	13 7	17 6	5 9	8 9	10 0	4 3	14 0	8 5
	U S A	10 3	18 4	7 6	19 0	28 3	22 0	18 5	37 2	22 5	23 9	17 6	9 2
Case fatality rate per 100 total notifications	E & W	16 2	18 8	9 1	13 0	11 0	9 7	8 3	7 6	7 4	6 8	4 3	2 4
	U S A	8 7	7 2	5 4	6 8	6 6	5 7	5 5	5 4	4 1	3 6	3 6	3 9†
Paralytic cases per 100 000	E & W						12 5	3 4	6 1	6 6	2 9	8 2	4 5
	U S A (approx)							11 9	23 3	12 7	13 8	9 2	4 9
Paralytic case D fatality rate per 100 paralytic cases	E & W						13 6	14 2	D 10 7	11 4	10 2	7 3	5 0
	U S A							8 6	(8 6	7 3	6 2	6 9	7 3

The figu for p alytc ses in USA are only e y app o im te they are l ul ted n the as umpt that th p px u n f p alytic to n np lyt c es r m ns unch g d n the n pecifi d notificat n

D—The pa lytic c e fat l ty rate th gr at D n h ej d m c of 1952 wa 10 7—th s e figur fo E gland nd W les f r that y

† Th s approx mate figu e b ed on 10 p r cent sample of d th certificate

Table 160 shows the notification of all cases in the United States and in England and Wales during the last 11 years and the fatality rate per 100 cases It is quite clear that the great variation in the severity of the epidemics in both the United States and England and Wales has had no appreciable effect on the steady reduction of the case fatality rate during these years

In the right part of the diagram (Fig 299) is the same information recorded with regard to paralytic cases In England and Wales the re

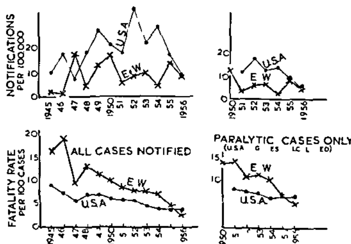


FIG 299 Fatality rate diagrams indicating all cases notified in England and Wales and the United States the notifications per 100 000 and the rate for paralytic cases only

Statistical Considerations of Mortality and Prognosis of Patients with Respiratory Disturbances

DR. W. RITCHIE RUSSELL

Twenty years ago the case fatality rate in some large outbreaks of poliomyelitis was more than 20 per cent of all paralytic cases, and clinicians will agree that the cause of death in the great majority of the cases listed must have been some form of respiratory insufficiency.

The methods of treatment developed in the past 20 years have changed this picture completely so that the paralytic case fatality rate should now in general be below 5 per 100 paralytic cases and in some large outbreaks has been brought down to a much lower figure. Thus, I think it should be mentioned that in Los Angeles County, California, in 1954 to 1955 there were over 1,000 paralytic cases, of which 175 required respiratory aids or tracheotomy and yet only 15 died—a paralytic case fatality rate of less than 1.4 per cent. This is surely a record, and one to be proud of.

This improvement in mortality began first in the United States in relation to the interest forced on the medical profession by the great epidemics in the 1950's. In some respects, the methods developed in the United States for saving life in acute poliomyelitis have until recently remained far ahead of those used in other countries of the world, thanks partly to the remarkable assistance in research provided by the National Foundation and partly to the disorganization of research in Europe brought about by the second great war.

To some extent, statistics now can be used to study these changes, owing to the surprising fact that during the past 10 years, in both the United States and Britain, the case fatality rate seems to have been almost entirely uninfluenced by the type and severity of the epidemics that have occurred.

Twenty years ago the case fatality rate varied considerably from year to year, but it seems that modern methods of saving life have abolished this variation to a large extent, as there is no evidence to suggest that the disease has become less severe. On the contrary these figures suggest that this proportion of life-threatening cases remains fairly constant from one outbreak to another and as in the great epidemic in Denmark of 1952, one quarter to one third of all paralytic cases are life-threatening in large outbreaks of the disease—I think, perhaps in all countries of the world. I think the idea that the stability of the outbreaks changes much from one outbreak to another is countered by the statistics of the last 10 years.

The steadily declining level in case fatality rates is simply an indication of improved methods of saving life during the acute stage. If this is so then we may conclude that the best and easiest indication of the standard of treatment in any one outbreak is provided simply by the case fatality rate for the paralytic cases in that outbreak.

Only in recent years have suitable statistics become available for such studies, and indeed the figures for England and Wales are the only ones that I have seen in which there is both an adequate separation of paralytic from nonparalytic cases and at the same time sufficient cases in each year for analysis and comparison of one year with the next. The Danish reports separate paralytic cases, but in many years they have listed few patients. Some of the United States reports separate paralytic cases from nonparalytic ones, but there is a large "unspecified" category so that for the whole country the paralytic case fatality rate can be assessed only approximately by assuming that the proportion of paralytic to nonparalytic cases in designated cases remains the same in those which are reported as "unspecified." This calculation has been incorporated in Table 160, and the figures from which this is prepared are published in the *Excerpta Medica* with which we all have been provided.

TABLE 160 NOTIFICATIONS AND CASE FATALITY RATES FROM 1945 TO 1956 FOR ENGLAND AND WALES (E & W) AND UNITED STATES (U S A)

POLIOMYELITIS		1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Total notifications per 100 000	E & W	2 0	2 6	18 1	4 3	15 7	17 6	5 9	8 9	10 0	4 3	14 0	8 5
	U S A	10 3	18 4	7 6	19 0	28 3	22 0	18 5	37 2	2? 5	?3 9	17 6	9 2
Case fatality rate per 100 total notifications	E & W	16 [illegible]	18 8	9 1	13 0	11 0	9 7	8 3	7 6	7 4	6 8	4 3	2 4
	U S A	8 7	7 2	5 4	6 8	6 6	5 7	5 5	5 4	4 1	3 6	3 6	3 9†
Paralytic cases per 100 000	E & W						1? 5	3 4	6 1	6 6	2 9	8 2	4 5
	U S A (approx)							11 9	23 3	12 7	13 8	9 2	4 9
Paralytic case fatality rate per 100 paralytic cases D	E & W						13 6	14 2	D 10 7	11 4	10 2	7 3	5 0
	U S A							8 6	[8 6	7 3	6?	6 9	7 3

Th fig es f r p alytic ca in U S A a nly v ry pp oximate they a e calcul ted n the a mption that th p p rt on f p alytic t o paralytic ca es rema n u chang d in the un pec fied notificat ns

D—Th paralytic e fatal ty ate in the g t Dan h p demic f 1952 was 10 7—the s me figur a for Engl d and W les for th t y

† Th approx m te figure b ed n a 10 per cent sample of d ath certificat

Table 160 shows the notification of all cases in the United States and in England and Wales during the last 11 years and the fatality rate per 100 cases It is quite clear that the great variation in the severity of the epidemics in both the United States and England and Wales has had no appreciable effect on the steady reduction of the case fatality rate during these years

In the right part of the diagram (Fig 299) is the same information recorded with regard to paralytic cases In England and Wales the re

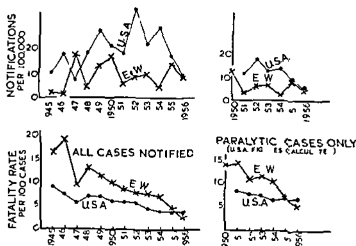


FIG 299 Fatality rate diagrams indicating all cases notified in England and Wales and the United States the notifications per 100 000 and the rate for paralytic cases only

liable recording of the paralytic cases began for the first time in 1950 and about the same time figures appeared in the United States but these have had to be calculated with the assumption which I have indicated so that they may not be clearly reliable But the figures for England and Wales are entirely reliable and the very steady reduction of the case fatality rate is a very striking feature In the Danish outbreak in 1952 the paralytic case fatality rate was 10 7 and it is interesting that the paralytic case fatality rate in England and Wales for that year was exactly the same—10 7

As the proportion of nonparalytic cases reported varies greatly from year to year for a variety of irrelevant reasons and as the diagnosis is in any case usually wrong it would seem that the *paralytic case fatality rate* is the most reliable guide with regard to the quality of treatment and yet the *total* case fatality rate for the earlier years is not without its interest

The steady decline in fatality rate shown in the foregoing Figure 299 is most gratifying and gives a vivid indication of the great advances in technic required to save life As far as Britain is concerned the research for this subject began intensively about 10 years later than it began in the United States and it was initiated by our great epidemic in 1947 The accelerated improvement of the past 2 years in England and Wales probably reflects the great interest and activity which followed the Danish outbreak of 1952 Dr Lassen s modes have not been approved everywhere but his provocative presentation acted as a great stimulus in Britain for which we are grateful Now we all use his methods for the combined bulbar and respiratory case and we now have plenty of reliable apparatus to maintain it for long periods The figures for England and Wales now rank with the best in the world and reflect great credit on both the organization now in operation by which life threatening cases are transferred to special centres and the physicians in charge of these hospitals many of who are here who have carried out the work involved

I am not one of those who approves of any form of nationalism in either science or medicine but perhaps it would be a good thing if there were some health rivalry with regard to getting these figures down in various countries In any case we in Britain may have a severe challenge ourselves this year as already the number of cases is running high and I am sure we will have your sympathy in our attempts to improve still further on our paralytic case fatality rate.

We all are looking forward to the disappearance of paralytic poliomyelitis and it is gratifying to know that the methods of artificial respiration and other methods which have been developed for poliomyelitis are proving now to be of great value in saving life in other diseases

Finally sir I am greatly indebted to Dr Bradley Dr Nathanson and Dr Landauer for providing figures on which these comments have been based but I rather believe that they would like me to add that they share no responsibility for interpretation

Control of Respiration

A. The Clinical Diagnosis and Evaluation of Respiratory Problems in Patients with Acute Poliomyelitis

DR. BJORN IBSEN

Justification for repetition in the presentation of fundamental subjects can be found in a change in approach as a result of recent developments in the treatment of patients with respiratory insufficiency. This results in changes in the evaluation of what is considered important with a bearing on indications for treatment.

Five years ago in Copenhagen during the big poliomyelitis epidemic the main problems in the clinical evaluation were in cases with extreme respiratory insufficiency. The technic of administering artificial respiration was so undeveloped that naturally there was hesitation in submitting patients to this active and dangerous treatment if they had a chance to survive on their own. With this in mind we postponed active treatment as long as possible with the consequence that when it was started there were already many of what we now consider late respiratory complications.

I was allowed to make a demonstration on an extremely ill patient, a 12-year-old girl who had paralysis of all four extremities, atelectasis of the left lung and a temperature of 104. She was cyanotic and sweating, drowning in her own secretions. A tracheostomy was done immediately under local anesthesia with insertion of a cuffed endotracheal tube. Endobronchial suction was done and the respiration controlled.

With the help of an oximeter of the Millikan type and Brinkmann's carbovisor it was easy to demonstrate the facts well known to anesthetists that with underventilation with 100 per cent oxygen it is possible to keep a normal oxygen saturation in the arterial blood and to have a CO_2 accumulation with its clinical signs of rise in blood pressure, sweating, restlessness and so on. This was the explanation for the condition of patients with underventilation in the respirators during oxygen therapy. A parallel could thus be drawn from Beecher and Murphy's work about CO_2 retention during anesthesia. The atelectasis could be treated, the airways protected and the circulatory problems handled.

A more recent patient—who was not a polio patient—was a 52-year-old man who was brought in after a traffic accident with a fracture of the skull, 10 fractured ribs on the left side and a rupture of the left lung with pressure pneumothorax and subcutaneous emphysema. Furthermore there was a rupture of the spleen. It was felt that this man would not be able to maintain sufficient spontaneous respiration after the removal of the spleen and that it would be too risky to let him try, so we did a tracheostomy and gave d-tubocurare for 4 days and administered artificial respiration. Then we stabilized the chest wall by traction and gave intermittent block of the intercostal nerves. On the 10th day the patient's spontaneous respiration was adequate and roentgenograms showed absolutely no changes in the lungs. Unfortunately this man died on the 14th day from a big pulmonary embolism on his second day out of bed. Other colleagues have had similar but more fortunate cases.

This case shows how much confidence we now can have in our active treatment as far as lung complications are concerned and demonstrates what consequences this will have in determining our indications for treatment. What I am aiming at is this—that the interest in the problems of clinical diagnosis and evaluation of respiratory problems has focused on the early signs where only a few years ago, at least in Denmark, we were more concerned about the late signs.

The more one puts weight on early signs the more one will be dependent on clinical diagnosis and clinical evaluation. That means in the old

Hippocratic way what can be seen heard or felt since this still is the main guide in spite of any improvement in lung function studies laboratory estimations x ray diagnosis and so on

To me the first clinical evaluation concerning respiratory problems in patients with poliomyelitis seems to deal with the criteria for the selection of patients in whom respiratory complications if not existing at present might be expected to develop and to give the indications for transfer of the patients to an intensive therapy unit or a special observation department where every means for observation and treatment shall be available including personnel as well as equipment

During the Copenhagen epidemic in 1952 we used the following as criteria for transfer (1) patients with marked cerebral involvement (2) patients with paralysis in the upper extremities (3) patients having difficulty in lifting the head (4) patients with rapidly progressing paralysis in the lower extremities (5) patients having difficulty in swallowing and (6) patients with paralytic cough

In 1952 we received 345 patients in this category

Two types of patients will arrive in the intensive therapy unit

1 The first type arrives at any stage of respiratory insufficiency They need immediate evaluation and immediate treatment

2 The other type arrives in time and immediate treatment does not seem indicated but eventual progression of symptoms must be followed in order to evaluate the right time for active interference

The observation must be intensive and a record should be kept to make it possible to follow variations in the temperature the pulse the blood pressure and the respiration since the clinical evaluation often must be based on changes more than on absolute levels

About the early signs I think that James Wilson has thrown light on their importance I do not expect to be able to say it in a better way so I shall quote him

The early symptoms of inspiratory involvement result from increased respiratory effort rather than from acute respiratory failure Many attempts have been made to use elaborate laboratory methods for the early detection of respiratory insufficiency specifically the determination of hemoglobin saturation levels and of blood carbon dioxide levels However in spite of respiratory muscle weakness increased effort in breathing may be successful in maintaining normal or even increased alveolar ventilation Therefore neither of these determinations nor any other blood chemical measurements now available are likely to prove useful in detecting early respiratory weakness and in fact may be misleading Determination of the reduction of vital capacity is helpful but in the absence of predetermined levels of the patient s normal capacity early and moderate reductions may not be detectable

Since Dickinson who works with Wilson is to be a discussant I shall not deal in detail with this very important matter

The most common classification of more marked respiratory disturbances has differentiated 3 categories (1) paralysis of the primary muscles of respiration (2) pharyngeal and laryngeal paralysis and (3) pathologic processes in the medullary respiratory centers

Although this classification is very useful it should be pointed out that mixtures of types in one patient are very common

What can be seen from this? The patient s respiratory state can be judged from his general appearance in terms of behavior and of skin signs as well as from specific respiratory activity The psychic signs to look for are anxiety restlessness fretfulness and emotional lability Pallor flushing cyanosis and sweating also can be looked for

Of respiratory action itself the frequency depth and rhythm should be evaluated One should look for use of accessory muscles flaring of the nostrils diaphragmatic or thoracic breathing and asymmetry of the movement of the thorax especially paradoxical respiration

In anesthesia I consider paradoxical respiration as a clinical sign which never should be allowed to develop However if it does it requires immediate active treatment In paradoxical respiration the ribs are drawn in during inspiration It may be symmetrical on both sides or more or less localized The causes can be classified as paralysis obstruction and atelectasis Sometimes they are more or less combined The clinical evaluation is very important since it has a bearing on the treatment

What can be heard? Respiration should be noseless When obstruction of the upper air

ways occurs increased effort is needed to draw the necessary amount of air through the narrowed passages giving rise to increased speed at the narrow place and thus more friction and increased noise This happens if the patient has sufficient muscle power to overcome the increased respiratory work This noise can be localized thus giving information about the site of the obstruction If the patient does not have sufficient muscle power—which very often is the case in polio—the noise is not an absolute indication of the degree of obstruction Sometimes the obstruction is nearly silent

The vital capacity can be estimated too if one asks the patient to blow to whistle or to count from 1 upward

Ask the patient to cough This cough can be paralytic or nonparalytic When the patient is coughing one can hear whether the cough is wet or dry This seems to me a main point in the evaluation of the kind of further management required Roughly speaking the outline can be as follows

1 If the cough is dry and nonparalytic there is time for observation

2 If the cough is dry and paralytic there might be indication for assisted respiration in a tank or cuirass

3 If the cough is wet and nonparalytic there is indication for a trial of postural drainage with possibly some suction and physiotherapy before the necessary tracheostomy is done

4 If the cough is wet and paralytic there is indication for tracheostomy and immediate treatment with controlled respiration in some way or another

What can be felt? The temperature and moistness of the skin can be felt The secretions in the bronchi can be felt easily and sometimes can be localized better this way than with a stethoscope The paralysis in the extremities can be felt and followed The pressure of the patient's forced expiration is a good clinical guide in determining the vital capacity

CONCLUSION

The severe polio cases present themselves with such wide variations in and complexities of symptoms that it is impossible to give definite rules for evaluation and treatment Each case has to be considered individually But it is worth remembering that nearly every case has parallels in clinical conditions familiar to any anesthetist whose job it is to watch and protect the vital functions sometimes under the most difficult conditions Since I am an anesthesiologist myself I hope you will excuse the fact that I look at the polio cases from an anesthesia point of view

What parallels can be drawn? First there is the unconscious patient who cannot maintain a clear airway Such a patient is similar to one in the poisoning center who has had too much morphine or barbiturate or to a patient with cerebral trauma or an intracranial vascular accident He is similar also from a respiratory point of view to the postanesthetic patient in the recovery room The unconscious patient cannot protect his own airways—postural drainage to avoid aspiration is essential and depression of respiration might call for stimulation or assisted ventilation The patient might be in shock and need fluid therapy along the usual lines

The patient with swallowing difficulties is known to the anesthetist in another way Patients with local analgesia in the throat after esophagoscopy or bronchoscopy must be protected against aspiration due to a lack of normal pharyngeal and laryngeal reflexes Patients with Wallenberg's syndrome in whom the 9th and the 10th cranial nerves are paralyzed are treated along the same lines

General paralysis may be due to extreme curarization after anesthesia or severe tetanus cases may have been converted by curarization into a resemblance to polio cases Myasthenia gravis patients in crisis or patients with a severe lack of potassium are both familiar to the anesthetist and are treated with his help Some polio patients are similar Polio cases with progressive paralysis of the lower extremities show the same problems as patients with high spinal anesthesia or patients with Guillain Barre syndrome who are treated by anesthetists

The anesthetist has to deal with patients with all types of pulmonary complications before during and after anesthesia He has to treat them and follow them and the same principles will be used in polio lung complications

Polio patients with ileus and with depressed respiration due to high lying diaphragms are similar to patients with ileus in the surgical

clinic in the potential danger of vomiting and aspiration resulting in interference with function of the lower lobes of the lungs forming compression atelectasis

The anesthetist is used to handling patients with tracheostomies anesthetizing them and cooperating in physiotherapy inhalation therapy and control of the conditions of the air around the tracheostomy

Poliomyelitis has done much to improve understanding of the proper management of patients with respiratory insufficiency This management and the clinical evaluation in polio does not consist of special problems but only of problems similar to the ones which are always found where respiration is impaired to a small or a high degree finishing with absolute respiratory insufficiency An attempt should be made to combine the treatment of all conditions of respiratory insufficiency along the same lines which have been developed so wonderfully through the vast experiences gained in polio work When polio incidence goes down let us use the co-operation of anesthetists who can form a pool of trained personnel and the respirator centers for treatment of any respiratory insufficiency As long as a patient cannot breathe sufficiently it does not matter much why since the immediate therapeutic challenge is overwhelming and the same in all cases to administer a sufficient exchange of air in one way or another When this has been done it can then be evaluated which symptoms were due to the disease itself and which were due to inadequate respiration The figures from the result of active treatment are so encouraging that the conclusion can be drawn that many of the symptoms previously thought to be due to the disease were due to improper management of the respiratory problems When adequate respiration is secured early with specialized technic we will not see many of the late signs of respiratory insufficiency

BIBLIOGRAPHY

Beecher H K and Murphy A J Acidosis during thoracic surgery J Thoracic Surg 19 50 1950

Whittenberger J L and Ferris B G Alterations of respiratory function in poliomyelitis, Am J Phys Med 31 226 1952

Wilson J L Treatment of respiratory difficulty (a Symposium on Poliomyelitis) Pediat Clin North America 1 70 1953

DISCUSSION

Dr Dickinson I have the utmost respect for Dr Ibsen and for the contributions he and his colleagues have made toward better medical care in poliomyelitis I have been unable to find any important arguments with the basic concepts presented in his paper and perhaps my time can be utilized most effectively by accepting Dr Ibsen's invitation to discuss in more detail the early signs of respiratory involvement in poliomyelitis

Dr Ibsen has delineated the major categories of respiratory difficulty occurring in the acute phase of this disease namely (1) paralysis of the respiratory muscles (2) airway obstruction from laryngeal or pharyngeal paralysis and (3) malfunction of the medullary regulating centers

Respiratory difficulty in a poliomyelitis patient can result from involvement in one or any combination of the basic areas just mentioned However separate discussion of these problems is advantageous

First let us discuss the progression of signs and symptoms resulting from progressive paralysis of the muscles of respiration The respiratory apparatus is designed to supply adequately metabolic needs during periods of violent exercise and consequently the normal person can produce a degree of pulmonary ventilation from 15 to 20 times as great as he needs in the resting state It is logical then to assume that extensive paralysis of the respiratory muscles must occur before one should expect respiratory failure to result and in the vast majority of cases this is true However signs of respiratory difficulty appear long before the point of respiratory failure has been reached Respiration in the resting person is normally carried out with little or no visible effort

This tremendous reserve in ventilation capacity makes effortless breathing possible As more and more neuromuscular units are rendered inactive by progressive acute poliomyelitis the remaining muscle fibers assume an ever increasing load until fatigue ensues In an effort to rest his fatigued respiratory muscles the polio patient begins to use the accessory muscles of respiration and as Drs Wilson Ibsen and others have emphasized a combination of decreased respiratory reserve fatigue and increased effort is responsible for the early clinical signs of respiratory difficulty or dyspnea in the acute polio patient with respiratory paralysis Oxygen lack and or carbon dioxide retention may not occur until respiratory muscle paralysis has become extensive and the clinical picture of dyspnea is clearly evident In fact we have shown that in many patients with early and partial respiratory muscle paralysis actual hyperpnea occurs in spite of this handicap

The specific signs and symptoms related to respiratory difficulty from paralysis of the respiratory muscles can be grouped into three categories in the general order of their appearance The first group includes those signs and symptoms related to decreased respiratory reserve fatigue and increased effort but not related in any direct way to actual respiratory failure These include weak cough interrupted staccato speech increased rate of breathing increased heart rate dilatation of the nostrils and use of the accessory respiratory muscles The next group includes those which may or may not be caused by actual respiratory failure In this group we find anxiety restlessness sleeplessness and disorientation A third group includes those signs and symptoms of respiratory failure Here we find cyanosis hypertension convulsions and coma Some of these manifestations are self explanatory and every physician easily recognizes them some need further discussion

Weak Cough The normal cough requires a breath several times the size of the average resting tidal breath and the weak cough associated with respiratory muscle paralysis is due to the patient's inability to take a deep breath and also may be due to selective paralysis of the abdominal muscles with a resultant lack of ability to exhale forcibly The weak cough of pharyngeal paralysis is due to the patient's inability to close off his airway at the level of the glottis This patient in effect blows rather than coughs The weak cough of respiratory muscle paralysis is in general normal in character except

for marked decrease in intensity As the vital capacity falls one would expect the cough to become progressively weaker

Interrupted Speech This is a useful sign of respiratory muscle paralysis and is characteristic of a sharp diminution in vital capa ity Because *of this diminution a patient even before he is* quite aware of his own deficiency may talk in a manner in which the use of only a few syl lables *is interrupted by an inspiratory effort* This obviously results from his inability to take a deep breath Adults on being engaged in an ordinary conversation may thus well demon strate the reduction of vital capacity In chil dren we have to resort to other technics It is useful to induce older children to count and to ask them how many numbers they can count in just one breath With the co-operation which is so frequent in patients with poliomyelitis and which distinguishes this condition from many other encephalopathies we are apt to get a useful response to our request Refusal to speak at all or to use only simple words such as yes no and water may clearly indi ate severe respiratory deficiency

Increased Rate of Breathing This sign may be due to worry or fear or to many other con ditions besides beginning respiratory muscle paralysis which is in fact one of the rarest causes The rate of respiration is unstable in most young children and in many apprehensive ill adults as well Seldom is this sign useful in evaluating early respiratory muscle paralysis

Dilatation of the Nostrils This is a sign which also is seen in a variety of conditions having nothing to do with poliomyelitis or even with such respiratory difficulty as rapid breath ing One commonly may see both dilatation of the nostrils and an increased rate of breathing in children with emotional disturbances or who may have been crying recently Fever alone may cause this sign *of dyspnea to appear In older* children in adults and in young children who are otherwise stable the appearance of this sign *however is presumptive evidence that respira* tory muscle paralysis or fatigue is becoming progressively more severe

Use of Accessory Muscles of Respiration The use of the accessory respiratory muscles such as the platysma and sternocleidomastoid is such striking evidence of respiratory difficulty that it might well be excluded from a list of signs and symptoms of *early* respiratory weak ness However demonstration of the use of these accessory muscles can be brought about by certain procedures when such use is not spon taneously evident By inhibiting the action of the diaphragm by splinting the belly with one s hands one can exaggerate the difficulty owing *to intercostal weakness and can bring about the* use of accessory muscles or dilatation of the nostrils or other evidences of mild dyspnea Likewise in case of diaphragmatic weakness splinting of the thorax with one s hands can bring about similar evidences of dyspnea not previously apparent

Anxiety Anxiety can be seen in any hos pitalized child or adult However as evidence of air hunger or of a feeling of inad quacy of pulmonary ventilation it is very important One can evaluate the symptom only in light of the whole situation and of an understanding of the patient Its persistence with reassurance with the comfort of the presence of the parents and with the absence of doctors and their *needles may be clear evidence of respiratory* difficulty or even respiratory failure although it may still be due to other conditions

Restlessness This may be due either to hy poxia or to carbon dioxide retention However restlessness combined with sleeplessness may occur in the absence of actual respiratory failure being rather a result of a combination of tre mendous fatigue and the need to keep awake in order to breathe We find that broken momentary sleeping episodes which alternate with wakefulness are an ominous sign of respir atory difficulty

Disorientation This also may be due to hy poxia or to carbon dioxide retention This sign of course can result from direct invasion *of the brain* by the virus or from cerebral in flammation directly related to the infection but having nothing whatever to do with the state of respiration However it i probably the result of respiratory failure far more often than is generally recognized One too easily concludes that the virus which attacks the central nervous system has by direct action caused disorienta tion and mental confusion and fails to recognize

that these symptoms may be a secondary by product of respiratory failure

Cyanosis Blueness is a traditional sign of hypoxia and one that is easily understood Little further comment i needed here except to point out that it is indeed a very late sign When cyanosis occurs with respiratory difficulty due to paralysis one can be sure that the end is not far off With the blue cyanosis of actual respiratory failure there should be no need for chemi al determinations to demonstrate such failure Cyanosis in poliomyelitis is of course not necessarily the result of muscle weakness alone One expects any deficiency in carbon dioxide excretion and in oxygen supply to be parallel This is not always true We may find oxygen lack to the point of cyanosis without accompanying carbon dioxide retention Such a situation is not produced by underventilation alone and clearly indicates disease within the lungs themselves such as pneumonia or atelectasis Patients who develop nearly complete obstruction of a large bronchus usually because of aspiration of mucus or saliva may suffer from oxygen lack without carbon dioxide retention Perfusion of this obstructed segment of lung by pulmonary circulation may continue Obviously hemoglobin in blood passing through this segment of the pulmonary circulation will remain unsaturated This results in a net unsaturation of hemoglobin which if it becomes sufficiently severe will result in cyanosis However carbon dioxide retention may not o cur in this situation since such retention in the obstructed area can be completely compensated for by increased excretion of carbon dioxide in the remaining lung tissue which is well ventilated In some cases the disturbance in the balance of these two gases cannot be explained by either of the mechanisms mentioned

Convulsions and Coma These are agonal and terminal evidences of hypoxia and do not need further discussion

We do not wish to imply through emphasis upon linical signs and symptoms that laboratory procedures cannot be of great help in evaluating the patient with progressive respiratory paralysis Although biochemical changes of failure do not occur early nevertheless carbon dioxide tension and oxygen saturation studies can be useful later These chemical studies are also important for proper management once respirator aid has been instituted

The most helpful laboratory test during early respiratory muscle paralysis is serial vital capacity determinations Clini al symptoms of decreased reserve rarely app ar before the vital capacity has dropped to somewhere between 75 per cent and 50 per cent of normal A decrease of this magnitude is easily recognized and an exact knowledge of the normal vital capacity for each patient is not essential In children the test can be particularly difficult since the use of a face mask or mouthpiece in an apprehensive child may so disturb him that co-operation becomes impossible Nevertheless after two or three trials most patients even though very ill will co-operate with the procedure without being disturbed and a progressive fall in vital capacity can be accurately followed

The practical question always arises When is the correct time for the initiation of treatment of a patient in a respirator? This cannot be answered in any certain and absolute way on the basis of any measurement which we can make such as with the spirometer nor can we give an absolute answer by clinical observation alone since emotional fa tors enter into the decision Generally the use of a mechanical respirator is indicated as soon as the paralysis has progressed to the point where the patient has developed dsypnea and is becoming fatigued from the effort of breathing There are two reasons for this (1) there is good indirect evidence that if a muscle is exercised to the point of exhaustion during acute poliomyelitis the ultimate recovery of that muscle is jeopardized (this concept as logically applies to the respiratory muscles as to the extremity muscles) and (2) it is important to institute mechani al aid or oxygen lack may further damage nerve cells invaded by the virus

Therefore we do not look upon the respirator solely as a device to prevent death from respiratory failure and we should initiate its use long before extreme dyspnea or clinical or laboratory evidences of respiratory failure actually occur The machine should be used at the time when respiratory muscle paralysis is first detected At

this time some evidence of dyspnea can be detected even if it is made evident only by inhibition of the action of the diaphragm or of the intercostals as described previously Only actual trial can determine in many cases how successful a respirator will be in producing rest I would agree with Dr Ibsen that the early use of a mechanical respirator before the patient has become completely exhausted may well drastically reduce the incidence of severe pulmonary complications in patients with respiratory muscle paralysis

Control of Respiration

B Technics for Providing Artificial Respiration The Tank Respirator

DR ROBERT M EIBEN

The tank respirator undoubtedly has been the most extensively employed mechanical device to provide artificial respiration for patients with prolonged respiratory muscle paralysis It apparently has long been recognized that cyclic changes of pressure around the body result in passive respiration This principle which ultimately resulted in the development of the tank respirator was suggested or applied in the management of patients with respiratory problems long before the present-day respirator was designed

The first tank respirator used in the management of patients with respiratory poliomyelitis was developed by Drinker primarily as a laboratory device Intertank pressure changes were accomplished by an electrically powered centrifugal pump The range of pressures obtainable with this instrument considerably exceeded that obtainable in the modern version which is operated by a motor driven bellows Few of the respirators in use in the country at the present time are capable of producing pressures lower than negative 30 centimeters of water and higher than positive 10 centimeters of water

In an attempt to make the tank respirator more universally applicable in the management of severe respiratory insufficiency positive pressure attachments have been added to the newer models The positive pressure dome fits over the head end of the respirator and can be engaged only when the tank is opened The Bennett positive pressure attachment permits the synchronous application of positive pressure by means of a face mask or a tracheotomy adapter This device can be employed to augment the pressures obtained within the tank Both attachments provide adequate support for the patient when the tank is opened for nursing and physical therapy procedures

Since its development the tank respirator has been used with some apparent reluctance by all physicians Although all would acknowledge that the machine is a valuable device there is not infrequently procrastination before the patient is placed in the machine Whether this concern is the result of anxiety over possible harmful physiologic effects or merely a manifestation of the basic conservatism of the average physician remains obscure If we accept the concept that exhaustion and inadequate ventilation are injurious to the poliomyelitis patient it follows that early respiratory support is necessary

Initiation of artificial respiration in the patient with acute poliomyelitis is usually accomplished as a result of the clinical interpretations of observations made on the patient Mild respiratory insufficiency may be manifested only by restlessness inability to sleep or unwillingness on the part of the patient to talk or to be otherwise disturbed Paradoxical breathing inability to count slowly to 10 and flaring of the ala nasi are manifestations of severe respiratory distress and are in themselves indications for immediate respiratory aid

Vital capacity measurements are of value in the demonstration of mild respiratory insufficiency and when followed closely may be used as a guide for the initiation of artificial respiration I suggest that the patient be introduced to the respirator when the vital capacity has fallen to approximately 50 per cent of predicted normal This rather arbitrary figure is preferred because preparation of the patient can be accomplished in an unhurried and reassuring manner If the patient fails to relax or to synchronize his own respiratory effort with the respirator it will be safe to return him to his bed Such early failure may at times prove indirectly beneficial

for the fact that the patient could be removed from the machine may decrease his anxiety regarding it and make him somewhat less fearful of subsequent preparation for tank respirator support

The patient should be placed promptly in the tank respirator if the vital capacity falls to 35 per cent Support in the respirator should be continuous except for brief periods when the patient s respiratory efforts are observed or when nursing procedures are carried out In general the cuirass respirator and rocking bed are not recommended for use in the acute illness The so-called lesser aids do not have the ventilatory efficiency of the tank respirator and requirements of the patient cannot reliably be met with their prolonged use

Once the patient is established in the tank respirator the major medical responsibility is to ensure the adequacy of the ventilation provided by the machine No small part of the management of the patient within the tank is the maintenance of an unobstructed airway The wide range of pressures available within the tank are under ordinary circumstances adequate for most patients On rare occasions particularly in the tracheotomized patient the intertank pressure must be supplemented by the synchronous application of positive pressure to obtain the desired pressure differential Generally intertank pressures of negative 15 to 20 centimeters of water and positive 5 centimeters of water will adequately ventilate most adults Observations that intertank pressures as low as 10 centimeters of water applied to the normal subject will over ventilate him dramatically emphasizes that changes in the elastic qualities of the lung and the thorax of the polio patient do occur

One cannot depend upon the patient s interpretation of the adequacy of ventilation for if he is alert it is likely that he will demand over ventilation whereas if his sensorium is clouded it is likely that he will be insensitive to air exchange which is totally inadequate There is no substitute for the actual measurement of tidal ventilation and minute volume The nomogram of Radford has proven extremely valuable as a guide in providing adequate respiration In use the recommended tidal ventilations determined are exceeded by approximately 10 to 15 per cent

When hyperventilation of the patient is recognized the pressure changes to correct this situation must be made slowly The patient with some remaining respiratory ability may unconsciously assist the respirator Tidal exchange measured under these circumstances will not obtain when the patient fatigues and if intertank pressure is not increased to compensate for this reduction inadequate ventilation will occur

The tank respirator cannot prevent the development of pulmonary complications The loss of an effective cough and postural influences on the drainage of the respiratory tree often result in areas of malfunctioning lung ultimately leading to areas of atelectasis and/or bronchopneumonia Recognizing this interference with the adequate drainage of the respiratory tree we change the patient s position frequently Most patients should spend at least several periods of time daily in the prone position in the tank respirator After a tracheotomy it is no longer possible to turn the patient on his abdomen in the tank respirator and the management of the patient cannot be accomplished entirely satisfactorily unless there is some way to provide positive pressure through the tracheotomy tube when the patient is placed in this position

The tank respirator interferes with the diagnosis of pulmonary complications because of the dependence of the clinician upon physical diagnostic criteria However measurements of ventilation often will indicate difficulties in respiration that could not be detected by the experienced clinician and decreased tidal exchange at previously established satisfactory pressures often precede roentgenographic evidence of atelectasis and pneumonic process

Although there is a significant incidence of pulmonary infection in the respiratory poliomyelitis patient it is not considered desirable to place patients routinely on prophylactic antibiotic therapy simply because they are placed in the tank respirators When pulmonary infections occur they should be treated vigorously with antibiotics and chemotherapeutic agents and therapy should be discontinued when resolution of the process has been demonstrated

Technics for intermittent deep breath have been developed and would appear to be of great value in preventing atelectasis Intermittent deep-breathing attachments can be attached to

individual tank respirators or used with several machines by employing a flanged fitting to exhaust air from the tank through one of the arm ports A series of deep breaths are accomplished by reducing the pressure within the tank to negative 35 or 40 centimeters of water

SUMMARY

The successful use of the tank respirator in the management of the patient with respiratory poliomyelitis depends to some extent on its being used by a group of persons who are aware of the value and limitations of the machine Artificial ventilation must be initiated early certainly considerably in advance of real respiratory distress Frequent turning of the patient is necessary to provide adequate drainage of the respiratory tree and to permit more even distribution of air in the lungs Adequacy of ventilation may be determined by measurement of tidal exchange Arterial blood studies remain the only certain way to demonstrate effective exchange of gases between the lungs and blood Respiratory assistance should be continuous during the acute illness and weaning from respiratory aids should be guided by measurements of the patient s respiratory ability

BIBLIOGRAPHY

Baldwin E Def Cournand A and Richards D W Jr Pulmonary insufficiency I Physiologic classification Clinical methods of analysis standard values in normal subjects Medicine 27 243 1948

Dickinson D G Poliomyelitis acute anterior *in* Conn H F (ed) Current Therapy p 45 Philadelphia Saunders 1954

Drinker P and McKhann C F The use of a new apparatus for the prolonged administration of artificial respiration I A fatal case of poliomyelitis J A M A 92 1658 1929

Drinker P and Shaw L A An apparatus for the prolonged administration of artificial respiration J Clin Invest 7 229 1929

Ferris B G Jr and Smith C W Maximum breathing capacity and vital capacity in female children and adolescents Pediatrics 12 341 1953

Ferris B G Jr Whittenberger J L and Gallagher J R Maximum breathing capacity and vital capacity of male children and adolescents Pediatrics 9 659 1952

Kelleher W H Maintenance of respiratory function in poliomyelitis and other neurological disorders Lancet 271 68 1956

Radford E P Ferris B G Jr and Kriete B C Clinical use of a nomogram to estimate proper ventilation during artificial respiration New England J Med 251 877 1954

Spencer W A (ed) Treatment of Acute Poliomyelitis pp 20 21 Springfield Ill Thomas 1956

Stimson P M and Hodes H L A Manual of the Common Contagious Diseases p 506 Philadelphia Lea and Febiger 1956

Whittenberger J L and Maloney J V Jr Physiologic factors in the use of the body respirator for impaired respiratory function Dis Chest 22 141 1952

Control of Respiration

B Technics for Providing Artificial Respiration Intratracheal Positive-Pressure Respirators

DR GÖRAN HAGLUND

The history of positive pressure respiration starts at the end of the 9th Century B C with the Jewish prophet Elisha as we can read in 2 Kings The first man on record who experimented with regular intratracheal positive pressure respiration was Andreas Vesalius who made such experiments in the middle of the 16th Century A D The first to describe an effective and in principle a modern positive pressure respirator was John Hunter the English surgeon in 1776 In the scientific literature published up to date more than 100 different positive pressure respirators have been described and recommended About half of them have been presented during the last 10 years

The purpose of all artificial respiration is to give the patient physiologically adequate respiration In most cases and situations the central problem is to secure an adequate and effective respiratory minute volume The alveolar exchange of gases is of course the fundamental of respiration but to secure reliable analyses of the alveolar gases is in our opinion clinically difficult Our biochemist Jorgen Lehman therefore worked out a routine control method with simultaneous measurement of the respiratory minute volume and a simple analysis of the carbon dioxide content of the mixed expiratory air Every nurse can perform such a control on the ward in about 5 minutes as she is checking the controls on the respirator and she can do it without disturbing the patient Professor Lehman s method has spared our patients thousands of artery and vein punctures Every respirator patient must be controlled with pH analyses etc but by using Lehman s control method laboratory tests can be kept at a minimum

The first demand made on an automatic positive pressure respirator is that the operator can adjust the respiratory minute volume exactly to permit this volume to remain constant Despite moderate variations in resistance as a result of accumulated mucus in the airways the condition of the lung parenchyma counterpressure from the abdomen and so on a first-class positive pressure respirator will provide the patient with a stable respiratory minute volume

The respiratory minute volume must not only be a constant volume from minute to minute from hour to hour from day to day but also at the same time an effective respiratory minute volume If the valve system on the respirator is pressure sensitive to permit the pressure changes in the patient s airways to initiate the changing of the valves from inspiration to expiration and back *as is often the case the respiratory minute* volume pressed into the patient s airways and lungs will be unchanged even if the resistance in the airways and lung parenchyma increases But the respirator frequency will increase compensatorily This means an increase in the ventilation of the dead space and a corresponding decrease in the alveolar ventilation In other words it means a stable but ineffective respiratory minute volume! The other way round a decrease in resistance will lead to a hyperventilating effect

For these reasons the automatic changer of the valve system of the positive pressure respirator preferably is separated from the inspiratory system The valve changer must give a uniform respiratory frequency and have a relative wide variability from 10 to 25 in adults and 35 to 40 or more in infants A stable frequency is a great comfort to *the respirator patient and* will spare him and the attendants many unnecessary alarms A constant respiratory minute volume with a stable respiratory frequency will ensure an effective respiratory treatment During such treatment the control manometer connected

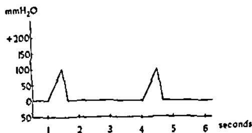


Fig 300 Type curve

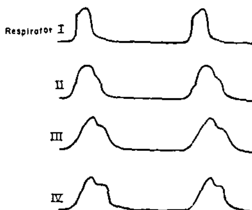


Fig 301 Pressure curves from 4 different respirators

with the patient s airways will give the well trained and observant attendant reliable and early clu s as to what is happening and it will incite him to proper prevention and treatment of threatening complications in the airways and in the lungs

The complex mechanism of the venous return and the pulmonary circulation during normal respiration and during positive pressure respira tion cannot be discussed in this paper It is sufficient to say that with our current knowledge we must insist on a pressure curve with a rela tively short inspiratory climb to pressure maxi mum and a still more rapid decrease to the zero line and with a relatively long pressureless pause before the next inspiration (Fig 300) We do not know for sure how to evaluate ration ally differences in pressure curves from different respirator models Four pressure curves from different respirators are shown in Figure 301 I beg your pardon that I don t have the zero line We see that the pressure curves are quite differ ent These curves are from a lung model and experiment One way of comparing different respirators is to measure the pressure masses defined as millimeter water pressure seconds per minute for each respirator during standard ized performance tests Such measurements from 4 Swedish respirators the 4 of which we saw the pressure curves are shown in Figure 30? We see that there are quite big differences among the pressure masses that these respira tors are giving

Opinions regarding a negative pressure phase after the passive expiration are also controversial My own clinical experiences indicate that such a suction may be a definite help in some cases with threatening non entral circulatory insuffi ciency Such a negative pressure is shown in the solid line of Figure 303 as compared with the non negative pressure curve with the dotted line Such a negative pressure will lower the abscissa of the inspiratory positive pressure and thereby give a decrease of the positive pressure masses Experiments have shown that the respiratory effect is unaffected by moderate negative pres sure phases We are experimenting further with the theory that a positive pressure respiration with negative pressure expiration where the positive pressure masses are (1) kept minimal for ne essary respiratory effect and (?) exactly counterbalanced by equal negative pressure masses shall give the optimal or the least un physiologic positive pressure respiration The effect of the negative pressure of 20 mm H O as illustrated in the figure was to decrease the positive pressure masses from 1 ?50 to 1 100 mm water pressure seconds per minute But the dif ference between the positive and the negative pressure masses became as low as plus 350 mm water pressure seconds

In endotracheal respiration most of the natu ral defense barriers against cold dry and dusty air are eliminated Therefore a positive pressure respirator must deliver efficiently prewarmed humidified and filtered air Tests have shown remarkable differences in these respects between different respirator models In some cases we have found dangerous defects

SUMMARY

The endotracheal positive pressure respirator should give a constant respiratory minute vol

PRESSURE MASSES

m ll mete water press re x seconds per minute

Min te Vol me		8 l ters	
F equency/m ute	16	18	20
Resp rato I	925	660	497
II	1060	935	640
III	1100	1015	885
IV	1490	1345	980

Fig 302 Differences in pressure masses in 4 different respirators



Fig 303 Pressure curves with and without negative expiratory phase

ume at a stable frequency The respirator should have a reliable mechanism for shifting of the valve system and for regulation of the different respiratory phases The respirator should have a device for negative pressure The air from the apparatus must be efficiently prewarmed humidified and filtered The respirator should have a manometer directly connected to the patient s airways

The technical construction should be simple all parts—especially the moving parts—should be capable of being easily inspected even when the respirator is in use and they should be simple to adjust and repair Unnecessary luxury and ingeniousness does not benefit the treatment nor the budget In clinical work lightweight and noiseless respirators are to be preferred All in all simplicity and tested reliability is what we need

Control of Respiration

B Technics for Providing Artificial Respiration Cuirass Respirators, Rocking Beds and Portable Respirators

DR GEORGE SAXTON, JR

In general cuirass respirators and rocking beds are less effective than the tank respirator or intermittent positive pressure breathing through a tracheotomy tube therefore they are used chiefly during convalescent and chronic stages of poliomyelitis with respiratory paralysis In other words whenever there are intrapulmonary complications such as increased secretions pneumonitis atelectasis or pneumonia these lesser aids should not be used However when the lungs are clear most patients can be given sufficient ventilation using cuirass respirators and rocking beds even when respiratory paralysis is complete and the vital capacity zero

A cuirass respirator can be thought of as an abbreviated tank respirator It produces passive inspiration by expanding the lower rib cage and drawing the diaphragms toward the abdominal cavity when a subatmospheric pressure is developed in the cuirass by a pump attached by a large hose as illustrated in Figure 304 However in order to be effective this type of device requires a good seal around the edge of the cuirass where it must contact the body Very little expiratory assistance can be achieved with this apparatus because the seal is broken by positive pressure in the range of 5 to 10 centimeters of water



Fig 304 Cuirass type respirator

The chief advantage of cuirass respirators is that they permit the patient to be free of the tank respirator or the tracheotomy tube Further more with certain shells which do not extend over the pelvis the patient may even sit up This then permits mobility in a wheelchair or automobile which is impossible in the tank respirator

However the fitting and application of a cuirass respirator requires some training on the part of the nursing staff and members of the patient s family In fact most patients with severe scoliosis cannot be fitted with a cuirass at all Also it requires some time for the patient to become accustomed to the cuirass and to tolerate it for 6 to 8 hours at a time These cuirasses should never be used 24 hours a day because the skin will usually break down under constant pressure

One of the unique values of the cuirass respirator is that it assists inspiration by producing negative intrapleural pressure and therefore probably aids venous return of blood to the right atrium However a disadvantage is that a cuirass limits the excursion of the upper ribs



Fig 305 Rocking bed with piston linked to motor



Fig 306 Synchronizing intermittent positive pressure breathing through a tracheotomy tube

during inspiration as can be demonstrated by fluoroscopy Of course the tidal volume which can be produced by a cuirass respirator is only about two thirds or 60 to 70 per cent of that which can be produced by a tank respirator in a given patient at comparable transpulmonary pressures

Dr Eve in England first described the use of the rocking motion in the resuscitation of patients suffering immersion or shock in 1932 The patient was placed on a board over a barrel or some such fulcrum and rocked back and forth In this way artificial respiration could be produced by the passive motion of the diaphragms resulting from shifting of the abdominal viscera About 10 years ago Dr Jessie Wright introduced the idea of mechanizing such a device to give patients with respiratory paralysis artificial respiration over long periods of time It has been used increasingly since 1950 in the United States so that in recent years many respirator centers have come to use this type of bed for their patients to the exclusion of regular hospital beds though the rocking mechanism may not be used more than 8 hours in 24 Patients find that they can eat read or even converse easily while rocking after they have become accustomed to this apparatus

The chief advantage of the rocking bed is that no equipment needs to be attached to the patient He need only lie down on the mechanism However it is the least effective of the various types of respiratory equipment moving even smaller tidal volumes of air in and out of the lungs than the cuirass respirator Therefore it is particularly well suited for use at night when the metabolic needs of the patient for gas exchange are least and the patient would not have occasion to be elsewhere than in bed anyway

There are a number of limitations to the rocking bed such as the fact that it produces motion sickness in about 10 to 20 per cent of



Fig 307 Intermittent positive pressure breathing at the mouth



Fig 308 Portable pressure breathing apparatus

patients at first This almost inevitably clears up with gradually increasing periods of use Furthermore body configuration is also impor tant in that a patient with a scaphoid abdomen often will not derive sufficient ventilation A certain abdominal avoirdupois is necessary to serve as a pendulum pushing and pulling on the diaphragms as a result of the rocking motion Infants and small children do not derive as much ventilation proportionately from rocking mechanisms as do adults

The rocking motion has a number of side effects in addition to ventilating the lungs For instance it can help a patient raise tracheo bronchial secretions in greater quantity than would be expected from the tidal volume produced However aspiration of secretions from the pharynx is a danger if there is bulbar in volvement Therefore the rocking bed should not be used as a ventilatory aid so long as pharyngeal paralysis persists

Some think that the rocking motion of such beds will increase cardiac output or at least stimulate cutaneous blood flow Others hope that the rocking motion might decrease the rate of demineralization of the skeleton which follows severe paralysis However these hy potheses remain unproven by measurements

One of the more interesting developments in the last 5 years has been the combination of the cuirass respirator with the rocking bed In 1957 Mr Emerson produced a few experimental models of a rocking bed with a piston linked to the electric motor which rocked the bed as shown in Figure 305 In this way a negative pressure in a thoraco abdominal cuirass could be produced through a hose connected to the piston during the foot-down position of the bed Tidal volumes produced by this combined action exceeded that produced by either mecha nism alone In practice this combination has proven useful occasionally in weaning patients from the tank respirator or intermittent positive pressure breathing through a tracheotomy tube It has been used almost exclusively for this transition to lesser aids for a few days or weeks at a time but not usually longer

Another combination tried more recently con sists of synchronizing intermittent positive pressure breathing through a tracheotomy tube with

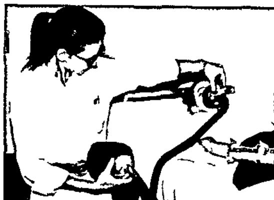


Fig 309 Portable hand bellows type of breathing apparatus

the foot-down position of the bed (Fig 306) This also is useful chiefly in transition from a major aid to a minor aid i e for introducing a patient accustomed to intermittent positive pressure breathing to the rocking motion

Until this past year most so-called portable respirators have consisted of rather heavy battery operated cuirass respirators These could be rolled about on a cart or dolly or transported in an automobile but they certainly could not be carried any appreciable distance However in 1957 a number of companies developed lighter units which can be carried

Two of these new portable units made by Monaghan Company and Thompson Company use a 15 pound or 25 pound blower instead of a heavy piston to lower the pressure in the cuirass A somewhat heavier power unit weighing 45 pounds has been developed by the Huxley Company for positive pressure in an inflatable abdominal binder called the Pneumobelt.

Another method of respiratory assistance which is portable has been coming into increasing use over the past few years It consists of intermittent positive pressure breathing at the mouth achieved by blowing air through a tube which the patient holds between the teeth In Figure 307 appears a vacuum cleaner which is marketed by the Emerson Company The patient merely closes his lips around the tube for inflation of his lungs and opens his mouth to permit expiration by elastic recoil of the lungs In this way the patient determines the depth and frequency of his breathing so that he can



Fig 310 Blacksmith bellows type of breathing apparatus

use it for an occasional deep breath and for lung stretching whenever he likes as well as for tidal ventilation It can also be used for a weak cough by elastic recoil of the lungs after maximum inflation Drying of the mouth from the continued blowing during expiration can be prevented by the patient s holding the tip of his tongue over the tube during expiration Patients often like to breathe with such an apparatus when they are being transferred from one type of equipment to another such as from a tank respirator to a chest respirator or rocking bed They can use it to good advantage when being transferred to another area of the hospital especially when the purpose is to get a roentgenogram of the chest made or to receive hydrotherapy Figure 308 shows the lightest portable weighing only 7 pounds which blows air continuously This is produced by the Thompson

Company and the patient holds the tube in his mouth for intermittent positive pressure breathing It can run on batteries or alternating current The ultimate in portable respiratory equipment is the hand bellows (Fig 309) which is produced by a number of different companies such as the Emerson Kriesleman and Monaghan Companies and last of all is the old fashioned blacksmiths hearthside bellows (Fig 310) which can be purchased through the equipment catalogs for only $5 and with an appropriate valve can do a good job of artificial respiration

Dr. Ferris The importance of an adequate ventilation should be emphasized since it is the primary concern of artificial ventilation The ventilation should not be too little the dangers of underventilation with carbon dioxide accumulation and lowered oxygen tension are well recognized Overventilation is also undesirable since the lowered arterial pCO adversely affects the functioning of the nervous system produces personality disturbances and makes it more difficult to wean patients from respiratory aids In our experience the use of the Radford nomogram has been a great asset in estimating the required ventilation Figure 311 presents a comparison of predicted tidal volumes from the nomogram and those actually measured with simultaneously collected arterial blood The measured tidal volumes have been corrected to a temperature of 24 C and an arterial pCO of 40 mm Hg since the nomogram was constructed for such conditions Here the mean difference is plus 3 04 per cent with a standard deviation of 21 5 per cent this comparison includes both acute and chronic patients using a variety of respiratory aids or breathing spontaneously Four of the 5 observations that are most out of line are the ones that are circled in Figure 311 which were obtained on patients who had known pulmonary disease complicating their poliomyelitis The nomogram is not suitable in such cases since there may be a low arterial oxygen saturation even in the presence of adequate removal of carbon dioxide In general the nomogram does permit a good prediction of the proper ventilation The pressures used in the breathing machines should be set to produce this ventilation I was pleased to hear that Dr Haglund and his associates have also used a bloodless method to estimate the adequacy of ventilation of their patients In general the addition of oxygen to the inspired air will not be necessary unless there is gross pulmonary pathology such as atelectasis or pulmonary edema

The next point to emphasize is the need to maintain a clear airway Frequently this can be done merely by the use of the prone or face down position This allows drainage of secretions from the mouth and minimizes the hazard of aspiration This position can be used in the body respirator but the required ventilating pressures are higher than they are in the supine position By this procedure tracheotomy may be avoided Frequent change of position is equally

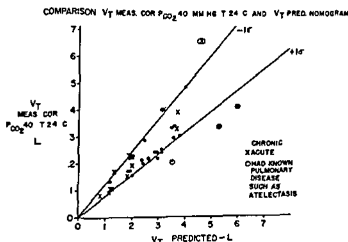


Fig 311 Comparison of predicted tidal volumes with those actually measured

important and must be done regularly whether the patient is in a body or tank respirator or is being respired by one of the other positive pressure breathing devices. This minimizes stasis within the lungs. It also helps to prevent pressure sores on the body.

If a tracheotomy is to be done, the largest size of cannula or endotracheal tube that can be inserted should be used to keep the resistance of the airway at a minimum. Any tube that is inserted into the trachea will be smaller than the trachea and the area available for air flow will be still smaller. Because of this its resistance is greater than that of the trachea and more work must be done either by the patient or the apparatus used for breathing to overcome this resistance. When the tracheotomy tube is no longer needed as a route for air exchange smaller tubes should be used. Since the patient is breathing around the tube even smaller tubes can contribute significantly to pulmonary resistance. Figure 312 shows the resistance measurements obtained on a patient with two different sizes of tracheotomy tubes in place and finally with the tube removed. The X's represent the mean value obtained and the lines plus and minus represent two standard deviations from the mean. The differences between the means of each group as tested by student's test demonstrate that the differences are significant at a P of less than 0.01.

The presence of a No. 3 tracheotomy tube (outside diameter 7 mm.) almost doubles the total pulmonary resistance. This tube is too small to breathe through easily and yet it contributes considerably to the resistance. Measurements on models have shown that this size tube in a tracheal model comparable to this patient's trachea increases the resistance threefold.

Much of our own work in the past few years has been directed toward assessing the efficacy of stretching the lungs and thorax by means of a deep breath. This technic was recommended in the early reports of the tank respirator. It was again suggested during the Minneapolis epidemic of 1946. Both of these technics used the maximum pressures developed by the tank respirator. Our investigations indicated that these pressures, usually about 25 cm. H_2O, were insufficient. In order to produce adequate pressures a booster in the form of a vacuum cleaner motor was used so that negative tank pressures up to 40 cm. H_2O could be reached.

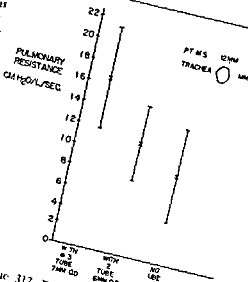


Fig. 312. Effect of tracheotomy tubes on pulmonary resistance.

The evaluation of this type of treatment involved measuring the compliance of the lung in the tidal volume range of patients with poliomyelitis. The effect of one or a series of deep breaths on their pulmonary compliance was studied. For comparison similar studies of the effect of shallow and deep breathing on the pulmonary compliance of normal persons were also made.

The results on a patient are presented in Figure 313. The initial lung compliance on the left is low. After two or more deep breaths in this instance produced by negative tank pressures of 30 to 35 cm. H_2O, there is an increase of 20 to 30 per cent in the lung compliance. If after a series of such breaths the lung compliance in the tidal volume range is followed serially there is a progressive fall in the lung compliance toward the initial control value. In the patients this appears to take place over a 1 hour to 2 hour period and it is faster when the patient lies in the semiright lateral position as shown to your right.

In a similar study on normal persons the control pulmonary compliance was measured in the supine position as indicated in Figure 314. Following this, the subject could be tilted by

means of a tilting bed to a sitting position Mid position change was prevented by using an inflatable balloon beneath an abdominal binder With the position change without lung volume change there is an increase in the measured lung compliance This we believe is due to an artifact introduced by the supine position However a few deep breaths produced an additional increase in lung compliance This increase also occurs when the subject lies in the right lateral position and in the sitting position even though lung volume changes are allowed to occur As in the patients the lung compliance decreases in these various body positions if the subject continues to breathe in a shallow or limited manner Here the change is more rapid than that seen in the patient with poliomyelitis

Because of the prompt reversibility of this phenomenon it is thought that the change in compliance probably is due to parts of the lung closing off and that these areas are reopened by sighing or taking a deep breath

The patient with poliomyelitis also should stretch his lungs either by means of various

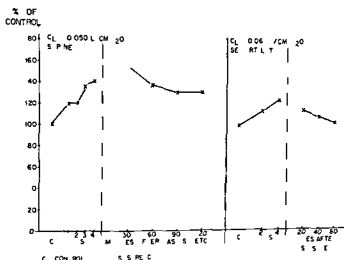


Fig 313 Per cent change in compliance of lungs as result of deep and shallow breathing in patients

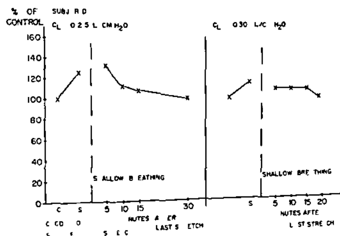


Fig 314 Per cent change in compliance of lungs as result of deep and shallow breathing in normal persons

mechanical devices or by using glossopharyngeal breathing

BIBLIOGRAPHY

Shambaugh G E Harrison W J and Farrell J I Treatment of the respiratory paralysis of poliomyelitis in a respiratory chamber J A M A 94 1371 1930

DR POSSI Having heard the reports on the different methods of artificial respiration in poliomyelitic respiratory failure we propose to analyze the problem as it arises in clinical practice notably from the viewpoint of the pediatrician I shall draw substantially on the experience gained at the Pediatric Clinic of the University of Zurich

We use four methods the clinical indications for each being quite precise in terms of on the one hand the symptomatology and on the other the stage of the disease i e whether it is acute or chronic

A statistical comparative evaluation of the results obtained with the different methods is impossible for a number of reasons First our extensive experience with the iron lung dates back to a time when our knowledge of pulmonary physiopathology was much less precise than it is today second each case of poliomyelitis—because of its clinical polymorphism—is different

TABLE 161 SIGNS OF RESPIRATORY INSUFFICIENCY

1 Increase in rate of respiration
2 Superficial irregular respiration
3 Diaphragmatic respiration
4 Inability to count to more than 3 or 4 without pausing
5 Reduced capacity for blowing
6 Reduction of the ability to cough
7 Pulsation of the nasal alae
8 Use of the auxiliary respiratory musculature
9 Anxiety
10 Tachycardia
11 Peribuccal cyanosis
12 Elevation of blood pressure
13 Drooling accumulation of secretions in the throat
14 Difficulty in swallowing liquids
15 Regurgitation of fluids through the nose
16 Nasal voice
17 Restlessness and insomnia
18 Confusional state
19 Coma shock

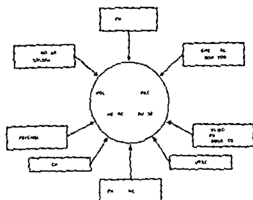


FIG 315 Medical team treating poliomyelitis patient in the acute phase of the disease

and cannot be compared with the others and third each apparatus has its advantages and disadvantages and therefore must be used according to the specific requirements of each case

In children the evaluation of a case and the conduct of the treatment are based above all on the clinical elements as ascertained by a team of specialists working in collaboration with the pediatrician (Fig 315) The laboratory findings are frequently of secondary importance because the possibility of repeated blood tests is rather limited

Analysis of the signs of respiratory failure requires experience and above all constant observation For this reason hospital personnel must be carefully trained to recognize the symptoms of respiratory failure (Table 161)

The simplest of clinical elements—such as changes in arterial pressure and in the electrocardiogram—seem to us especially sound criteria for recognizing any increase in the CO_2 level of the blood or respiratory distress Experience has shown the electrocardiographic appearance of signs of right heart strain when using the Drinker tank respirator signs which disappear when a positive pressure respirator is substituted

The choice of the apparatus to be used in the acute phase depends on the severity of the respiratory failure the extent of paralysis and the tendency of the respiratory passages to obstruction We use the iron lung when the signs are mild and when there is no respiratory obstru

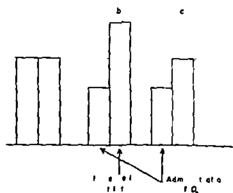


Fig 316 Oxygen—carbon dioxide balance in various pulmonary and cardiac complications in poliomyelitis patients

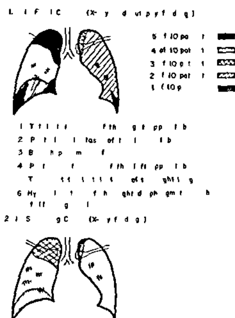


Fig 317 Frequency of pulmonary complications in patients receiving positive pressure artificial respiration

tion In more severe forms for example when bronchial rales appear we favor tracheotomy and a positive pressure respirator

Again it is clinical observation which enables us to detect modifications in the O CO equilibrium and to select the appropriate therapy (Fig 316) The appearance of respiratory insuf

Dying

Case	Name	Age	No
1	B E	16 y s	3643/54
2	S A	5 y s	4844/54
3	R R	12 yrs	5051/54
4	A.H.	4 yrs	5387/54
5	W W	5 y s	4535/54
6	Z O	6 y s	4749/54
7	B R	7 y s	4771/55
8	W G	16 yrs	7200/55
9	M B	9 yrs	7161/55
10	H.B	7 y s	4499/57

Surviving

Case	Name	Age	No
11	Z J	5 y s	4064/54
12	B H	5 yrs	7249/55
13	H H	8 y s	4941/55
14	H R	3 yrs	8386/56
15	N B	2 y s	6326/56
16	K E	4 y s	9431/56
17	S W	10 y s	1068/57
18	T E	14 y	1030/57
19	R R	14 yrs	547/56
20	H E	15 yrs	1098/57

Time scale: 0 at o 1 2 3 4 5 6 8 10 12 mo ths

Pathological ECG

Fig 318 Cases of poliomyelitis receiving positive pressure artificial respiration at the Zurich Kinderspital (Children s Hospital)

ficiency as well as obstruction of the airways produce a drop in the oxygen saturation blood level and an accumulation of CO Administration of oxygen corrects the hypoxia while an increase in ventilation permits the elimination of CO However the occurrence of pulmonary edema or cardiac insufficiency will require only administration of oxygen

The choice of the method to be used does not depend solely on the degree of respiratory failure or the state of the respiratory passages it also depends on the general clinical signs of the illness In cases where peripheral paralysis is very pronounced requiring intensive physiotherapy and frequent changes of position the positive pressure respirator is indicated because it has undeniable advantages in such cases The same applies to cases in which hygienic care and control of the course of the disease (pulmonary and cardiac auscultation repeated electrocardiograms etc) are such as to call for a simple approach (See Table 162)

Table 162 Indications for Use of Iron Lung and Positive Pressure Respirator

	Iron Lung	Positive Pressure Respirator
1 At the onset of respiratory insufficiency	+++	
2 When the respiratory passages are clear	+++	+
3 When the respiratory passages are obstructed by mucous secretions	+	+++
4 Physiotherapy	+	+++
5 Hygienic care	+	+++
6 Control of the course of the disease	+	+++
7 Psychological effect	+	+++

I join Haglund in insisting on the necessity not only of heating but also thoroughly humidifying the air used This was achieved by increasing the temperature of the water in the Engstrom apparatus With us too the tidal volume range was determined with the aid of a Radford nomogram Our experience has shown—and this confirms the view of Liben—

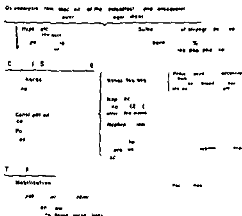


Fig 319 Disturbances of calcium metabolism due to immobilization

that the figures must be increased by 10 to 20 per cent especially in the case of very young children The problem of antibiotic prophylaxis was solved in our cases by systematic administration of a broad spectrum antibiotic which must be selected and if necessary changed according to the findings of the sensitivity tests (antibiogram) We feel that such prophylaxis must be kept to a minimum and discontinued as soon as possible However it must be reinstated at the slightest indication of pulmonary complication

It has been claimed that positive pressure respirators cause atelectasis especially of the upper lobe more frequently than does the iron lung or Drinker respirator (Fig 317) Various hypotheses have been postulated on the physiopathogenic mechanism of this complication If we mention it today it is primarily to emphasize the importance of procedures designed to drain the bronchi postural drainage and of systematic and repeated clearance of the bronchial tree which is greatly facilitated by the use of positive pressure respirators It should not be forgotten that pulmonary complications occur not only during the acute phase but also during the subacute and the chronic phases of the disease

In the chronic phase respiratory failure proper becomes less important and it is not as necessary to keep a close and repeated check on the O/CO equilibrium On the other hand cardiac repercussions—and I am thinking above all

of the pulmonary or right heart—pulmonary complications psychological hygiene electrolytic changes and more especially disturbances in calcium metabolism are assuming ever greater importance (Fig 318)

We could not confirm in our patients the occurrence of pulmonary emphysema as constantly observed by Haglund I would be interested to find out on the basis of what criteria Haglund formulates a diagnosis of emphysema? According to our experience the appearance of calciuria—an expression of decalcification of the organism due to inactivity—does not seem to be affected by use of the rocker bed Anyway this point will be the topic of tomorrow's discussion (Fig 319)

In conclusion we think that the choice of a method of artificial respiration depends less on the theoretical value of the method as such than on the exact clinical evaluation of the case to be treated During the course of the disease it is frequently advantageous to switch from one method to another depending on the requirements of the moment

Care of Patients Severely Stricken by Poliomyelitis

THURSDAY MORNING, JULY 11, 1957

(This Session Convened in the Aula of the University of Geneva)

Chairman

DR. ELIAS BENGTSSON
Epidemic Hospital
Stockholm

Speakers

DR. FRED PLUM
School of Medicine
University of Washington
Seattle

DR. WILLIAM A. SPENCER
Southwestern Poliomyelitis
Respiratory Center
Jefferson Davis Hospital
Houston

DR. INGEMAR JUNGNER
Epidemic Hospital
Stockholm

DR. HANS NORDENSTAM
Hospital for Infectious Diseases
Stockholm

DR. HAROLD N. NEU
Creighton Memorial
St. Joseph's Hospital
Omaha

DR. AVRON Y. SWEET
The Mount Sinai Hospital
New York

DR. LEONARD V. WENDLAND
Rancho Los Amigos Hospital
Hondo, California

Discussants

DR. CARL AARSVOLD
Ulleval Hospital
Oslo

PROF. DR. PETER HOLST
Ulleval Hospital
Oslo

DR. LEON LEWIS
Fairmont Hospital
San Leandro, California

DR. T. NEUKIRCH
Blegdamshospitalet
Copenhagen

DR. MANUEL C. SANZ
University Hospital
University of Geneva
Geneva

Emancipation from the Respirator Following Poliomyelitis

DR FRED PLUM

Improved treatment of respiratory failure caused by acute poliomyelitis has produced a sharp reduction in the disease s mortality rate Where once 90 per cent or more of the patients placed in respirators succumbed at least 8 out *of 10 will now survive their acute illness and* it is rare to lose a patient once the acute stage of the disease is over Such success in treating the acute phase is gratifying but has created the problem of the rehabilitation of persons *with serious permanent limitations in breathing* function

The present communication summarizes experience with a program of emancipation from respiratory aid employed for the 70 patients who have been discharged from our own center in Seattle Washington

Nearly all these patients came in tank type respirators Although cumbersome and often inconvenient the tank provided a greater respiratory reserve than did chest respirators or rocking beds However once the acute illness had passed a patient s ventilatory demands usually decreased rapidly so that emancipation to lesser respiratory aids or to respiratory independence could be started promptly

Successful convalescent regimens are going to vary in specific methods depending upon the experience and preferences of different staffs Our own program s paramount aim was to assist the patient in developing maximally *all* his *residual abilities so that he could pursue as far* as his powers permitted a normal way of life encumbered as little as possible with mechanical devices of any type Total emancipation from artificial respiratory aid although emphasized in this program was subordinated to this more comprehensive goal of rehabilitation

Although all patients were eventually freed from depending upon the tank respirator not all patients were able to dispense completely with respiratory aid after poliomyelitis Many factors influenced the rate and degree of recovery but the most important were the degree of damage to the peripheral and central respiratory neurones the level of general health the amount of pulmonary and thoracic complications the level of ventilation both in the tank and in lesser aids and finally the psychological attitudes of the patient Since these five factors were so important in determining the ultimate therapeutic results they will be discussed in some detail

The degree of damage to peripheral and central respiratory neurones Since the muscular act of breathing is complex it is usually only crudely analyzed For clinical purposes the respiratory function of each patient was evaluated in terms of vital capacity diaphragmatic activity intercostal activity and whether or not accessory muscles were employed during quiet breathing These tests have been shown to provide an accurate functional appraisal of ventilatory capacity No patient became completely emancipated from artificial respiratory aid when he still used accessory muscles to carry on quiet breathing In general complete emancipation from artificial respiration during sleep was impossible until over 20 to 30 per cent of the predicted normal vital capacity had returned Whether a patient with a vital capacity between 25 per cent and 50 per cent became completely free of the need for respiratory aid at night depended on two things first the distribution of residual functioning neuromuscular units and *second the status of central respiratory control* Patients whose residual respiratory muscles were largely diaphragmatic breathed with less effort and became free of the respirator at lower vital capacity levels than did patients whose residual breathing was largely intercostal in origin

Two patients with bulbar poliomyelitis showed evidence of permanent defects involving their central regulation of respiration The upper two tracings in Figure 370 are the pneumograms of these subjects who had great difficulty in sleeping without artificial respiratory aid despite maximal breathing capacities of over 30 per

cent and 50 per cent of the predicted normal When they did become sufficiently fatigued to sleep they demonstrated irregularity in respiratory rhythm abnormal retention of carbon dioxide with pACO as we see here ranging on the one hand to 55 4 and on the other hand to 50 ? and choreiform activity of the head and extremities Additional studies demonstrated low responsiveness to carbon dioxide as a respiratory stimulant and in addition more than 20 per cent reduction in ventilation while breathing 100 per cent oxygen A larger group of subjects as illustrated in Figure 321 also showed this low responsiveness to CO as a respiratory stimulant and these are findings which are similar to those observed by Dr Bengtsson in Stockholm ? years ago However the very flat curves of the two subjects AW and FL probably represent central damage rather than merely increase in the work of breathing

The second major factor then influencing the rate of recovery was *the level of general health* Emancipation to lesser aids or to progressively *longer periods of independent breathing* was seldom pressed unless patients were afebrile and over the critical autonomic lability which accompanied acute poliomyelitis Gastric tubes and swallowing paralysis did not deter emancipation efforts although trials on rocking beds or in cuirass or positive pressure respirators were delayed until several hours after feeding Caution was required with a few patients suffering from prolonged swallowing paralysis as they easily developed gastric distention and esophageal regurgitation when fatigued or frightened Of ourse aspiration of gastric contents was then a threat

The third major category was that of *pulmonary and thoracic complications* Respiratory infections created insidious and sometimes severe threats during onvalescence Merely a common cold caused the vital capa ities of many patients

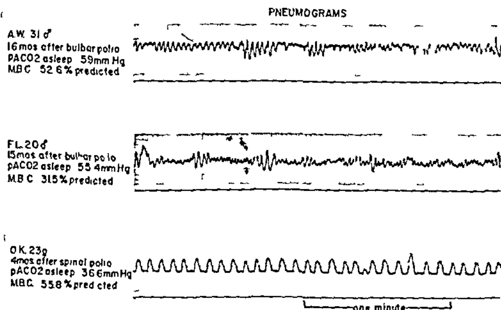


Fig 320 Chest pneumograms in two patients (AW and FL) with permanent irregularity in breathing following poliomyelitis In piration rea ls up The lower tracing demonstrates the chest pneumogram during sleep of a patient who had had spinal poliomyelitis with respiratory failure The irregularities in respiratory rhythm and abnormally high pACO in AW and FL were observed only during sleep when the above tracings were made

to fall 20 per cent to 30 per cent below their best previous efforts Tracheobronchitis created more serious problems and two patients with laryngeal paralysis developed obstructing sub laryngeal edema as the result of their ineffective barking coughing activity Therefore colds bronchitis and other pulmonary infections were treated vigorously in these patients Those who had mastered glossopharyngeal breathing to the extent of developing a cough proved to have the most effective protection against respiratory in fections When a cold developed respiratory aid had to be increased for almost all patients who were using respirators during any part of the day Some subjects recently freed from all re spiratory aid had to be returned to rocking beds or cuirass respirators particularly at night for the duration of the infection Most patients were placed in postural drainage while rocking Antibiotics were of less value than steam inhala tions postural drainage and inhaled nebulized bronchodilators or wetting agents Gentle tracheobronchial suction was reinstituted for patients who still had tracheostomies

Few tracheostomies were closed in these pa tients if they regained less than 35 per cent of their predicted normal vital capacities How ever metal tracheotomy tubes were removed as *promptly as possible in convalescence and re* placed by the Teflon plugs which we presented

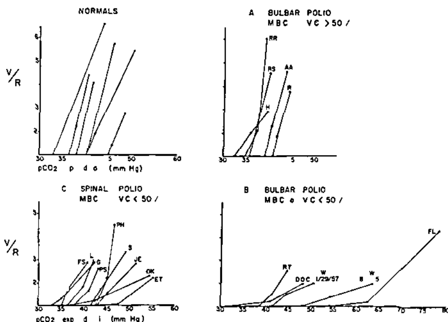


Fig 371 The ratio of evoked minute alveolar ventilation with CO_2 to resting minute alveolar ventilation (V/R) with air is given on the ordinate The $pACO_2$ is recorded on the abscissa Subjects breathed room air and mixtures of 3 per cent and 5 per cent CO_2 in air for 20 minutes from a Tissot spirometer The $pACO_2$ was measured at the mouthpiece with an infrared gas analyzer No patient had an MBC or VC of less than 25 per cent Normals doubled resting ventilation ($V/R = 2$) with a rise in $pACO_2$ of 1 5 to 2 0 mm Hg Group A doubled ventilation with a mean $pACO_2$ rise of 1 88 $\pm$ 37 mm Hg The mean rise in $pACO_2$ associated with $V/R = 2$ was 12 0 $\pm$ 1 70 mm Hg in Group B and 5 14 $\pm$ 1 36 mm Hg in Group C Fourteen of the above subjects also were tested for *their response to* breathing oxygen Although several showed moderate reduction on oxygen only A W and F L (in B) had more than a 20 per cent reduction in minute alveolar volume on 100 per cent oxygen

in our exhibit in the other building These plugs eliminated the irritation of the trachea were cosmetically more attractive and contained an obturator which could be removed immediately to provide access to the trachea

Pulmonary infections and tracheobronchitis impelled increased caution in respiratory emancipation but did not prevent freeing patients from the tank respirator Actually in several instances infections could be handled better on the rocking bed than in the tank respirator since rocking with the patient lying prone provided needed pulmonary drainage Figure 322 shows a young man who had been in the tank respirator for 4 months during which time he had approximately 25 bronchoscopies These had had the success which may be seen on the left side of the chest It was possible to get him out of the tank and place him prone on the rocking bed Therefore it was possible by the simple return to gravitational and postural methods to clear this completely while at the same time moving him from the body respirator

A significant factor impeding the rate and degree of respiratory recovery was the reduced chest lung compliance suffered by many patients This increased chest resistance required greater than usual effort or higher respirator pressures to expand the lungs and sometimes accounted for difficulty in obtaining adequate ventilation with certain types of chest respirators The treatment of this complication has been discussed at length by others suffice it to say that in our own experience glossopharyngeal breathing was the only effective way of overcoming the problem of increased pulmonary thoracic compliance

The fourth factor modifying the rate and degree of recovery was the *level of ventilation in the tank respirator and in lesser aids* As has been emphasized many patients in tank respirators were comfortable only when hyperventilated This began during the acute stage of the illness even before the respirator was used and if sustained created a greater ventilatory demand than could be met by chest respirators rocking beds or other assistive devices Therefore measurement of tidal volumes and minute volumes in respirators was carried out constantly

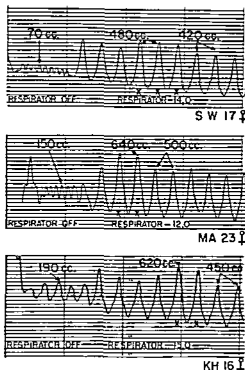
Finally and perhaps in many ways more important the *psychological factors* modifying



Fig 322 P A chest roentgenograms on patient R O C The upper roentgenogram (5/22 54) showed changes which reportedly had been present for 3 months He was placed prone and in postural drainage on the rocking bed and copious amounts of secretions presented at the tracheotomy for removal (*Bottom*) By 5 28 the major part of the atelectasis had cleared

the rate and degree of recovery were considerable Some patients were much more apprehensive than others over being removed from the respirator Patients who had suffered periods of anoxia or those whose previous management had been marked by inexperience or uncertainty were particularly frightened by emancipation efforts It was seldom fruitful to press the apprehensive patients a ceptance of emancipation procedures Such forced therapy slowed the total recovery process Engendering self-confidence in the patient sometimes required con

ASSISTED VITAL CAPACITY IN RESPIRATOR PATIENTS



X—RESPIRATOR—ASSISTED VITAL CAPACITY

Fig 323 The relationship of assisted vital capacity to unassisted vital capacity in respirator patients Tracings read from right to left In each of the three instances illustrated the AVC was measured as described in the text The respirator was then turned off in these 3 co-operative patients and their unassisted vital capacity was recorded *S W had an AVC of 60 cc* a spontaneous vital capacity of 70 cc M A had an AVC of 140 cc a spontaneous VC of 150 cc K H had an AVC of 170 cc a spontaneous VC of 190 cc

siderable time but usually succeeded in restoring to the patient rather than to the staff motivation for recovery

Evaluation to determine the patient s ability to transfer from tank or positive pressure respirator to lesser aids was performed as soon as the acute illness or severe pulmonary complications had passed Measurements of breathing in the respirator were made at frequent intervals with a spirometer to detect and correct hyperventilation Next the extent of residual respiratory function was determined

A common method of determining the amount of intrinsic breathing ability remaining after acute polio is that of opening a respirator portal and visually examining breathing efforts This is not quantitative particularly at small respiratory volumes and sometimes frightens the patient We have found the measurement of what we call the assisted vital capacity to be preferable for estimating quantitatively residual ventilatory ability The assisted vital capacity is performed without opening the respirator and therefore is particularly useful when dealing with apprehensive or seriously paralyzed patients The tracings in Figure 323 read from right to left and consist first of resting volume in the respirator followed by a maximal deep breath over the respirator s tidal volume followed in these three co-operative subjects by the measurement of spontaneous unassisted tidal volume or vital capacity The patient is able to *increase over the respirator tidal volume* almost as much or exactly as much as he is able to achieve on his own when unassisted by the respirator This was borne out by consecutive trials on over 15 patients for none of whom was the standard error greater than 15 per cent and in whom the average deviation was approximately 12 per cent. Not until the spontaneous vital capacity rose to over 500 to 600 cc did the assisted vital capacity deviate more than approximately 20 per cent from the actual finding in unassisted patients (Table 163) Patients whose assisted vital capacity was 15 per cent or more of their predicted normal vital capacity were *rapidly and easily liberated from* tank respirators whereas patients whose assisted vital capacity at the end of the acute stage of poliomyelitis was 5 per cent or less of the predicted normal (Table 164) usually required more cautious transfer to rocking beds or chest respirators

To turn these to a specific program the first step in the emancipation from the respirator was accustoming the patient to ventilation by means of a positive pressure respirator or cuirass This was done with the patient kept on the cart of the tank so that if difficulties arose immediate return to the body respirator could be effected

Patients who demonstrated an AVC of greater than 10 per cent to 15 per cent of the predicted normal or who could breathe spontaneously from 3 to 5 minutes were transferred directly from

TABLE 163 COMPARISON OF ASSISTED VITAL CAPACITY IN RESPIRATOR PATIENTS TO EARLIEST MEASURABLE UNASSISTED VITAL CAPACITY

Name	Age	Sex	Resp Tidal Volume	Resp Vital Capacity	Assisted Vital Capacity	Unassisted Vital Capacity	% Difference
F A	15	F	380	500	120	130	—
K H	16	F	450	620	170	190	7.1
P S	17	F	470	800	380	440	−13.6
S W	17	F	440	510	0	0	0
R O C	22	M	480	950	470	540	13.0
L B	23	F	500	940	440	440	0
H S	24	M	530	930	400	420	−4.8
V B	26	F	460	520	60	0	14.3
R R	28	M	640	1840	1200	1440	16.6
M A	29	F	500	640	140	140	0
R S	30	M	500	1240	740	800	5
H A	43	F	340	760	420	480	12.5
						Mean	8.1%

the tank to a hospital or a rocking bed. However, as a rule, they were first familiarized with positive pressure breathing. It proved easiest to adapt patients to a cuirass or positive pressure respirator first and then work from this to progressively longer periods on the rocking bed.

The choice of which respiratory assistive device to employ for the convalescent patient depended upon his residual respiratory function and on his activity. We preferred to use rocking beds for sleeping and resting. Chest respirators or positive pressure devices were employed for activities such as sitting, Hubbard tank physiotherapy or ambulation. The rocking bed provided most patients with a great sense of personal freedom. Its motion was comfortable and stimulating and it eliminated the need for much of the repeated moving and positioning which paralyzed patients otherwise required. Most patients learned to rock while lying prone which facilitated pulmonary and renal drainage. As improvement took place the bed was employed for sleeping and other respiratory devices were progressively eliminated during the day time.

The two factors which most impeded the elimination of respiratory aid were the number of irreversibly damaged anterior horn cells and the reduction in chest lung compliance. Although we specifically avoided making conva

TABLE 164 RECOVERY OF VENTILATION TRACED BY ASSISTED VITAL CAPACITY

Patient	Date	Resp Tidal Volume	Resp Vital Capacity	Assisted Vital Capacity	Unassisted Vital Capacity
K H	12/22/53	540	630	80	Unobtainable
16 F	1/1/54	480	600	120	Unobtainable
Acute	1/8/54	510	620	110	Unobtainable
onset	1/14/54	530	660	130	100 (approx)
12/24/53	1/22/54	450	610	160	190
	2/15/54	440	800	360	3 0
M A	7/22/54	440	5 0	80	Unobtainable
23 F	7/23/54	490	600	110	Unobtainable
Acute	7/25/54	500	640	140	140
onset	7/30/54	5 0	700	150	160
7/5/54	8/9/54	480	0	40	-40

lescence an endurance contest all patients were maintained on a program of progressively increasing their independent breathing times to tolerance Other therapeutic methods designed to strengthen the respiratory muscles were extremely disappointing Occasionally mastery of the technic of effective glossopharyngeal breathing was followed promptly by a rise in vital capacity of 30 to 50 per cent but this probably reflected increased chest compliance rather than improvement in strength of breathing muscles

It was our practice never to force to exhaustion those patients who claimed they needed assistance to sleep without respiratory aid no matter what the measurement of their vital capacity Gradually reducing respirator pressures or rocking bed during sleep helped a few patients in overcoming the need for nocturnal aid but it was usually more successful to wake the patient at 4 A M turn off the respiratory device at that time and urge him to return to sleep The dark and quiet ward the recent sleep and the knowledge of constant supervision reduced to a minimum stimuli which otherwise would contribute to arousal

Although our major emphasis in the paper has been on a program for respiratory emancipation it should be stressed that our goal in the rehabilitation of patients with respiratory insufficiency was more encompassing than the elimination of respirators Some patients never recover enough breathing function to become completely independent of respiratory aid Others were marginal and it was found that they were capable of much more active daytime programs if they were assisted at night with artificial respiration

Many of the patients who are referred to a respirator center are sent from other hospitals many months after illness because they have failed to become free of respiratory aid In many instan es elimination of all use of the respirator has been set as the foremost goal of therapy Having failed to reach this goal these patients have suffered a deep sense of defeat and a long period of transition is required to redirect them toward potentially achievable objectives However once they accept the principle that a respirator is no different from any other crutch many of them can make remarkable strides Seventy patients have been discharged from our unit in the past 3 years 18 still require partial respiratory support but none require a tank respirator and most use a rocking bed only while sleeping Despite their partial dependence upon artificial respiration 12 of these 18 are actively engaged in either schooling housework or gainful employment All are home and all are active socially These are encouraging results and justify the care and effort required to develop a comprehensive program of respiratory emancipation and rehabilitation

BIBLIOGRAPHY

Affeldt J E Bower A G Dail C W and Arata N N The prognosis for respiratory recovery in severe poliomyelitis Arch Phys Med & Rehab 38 290 1957

Brahdy M B Late results of 63 cases of poliomyelitis treated in the respirator New York State J Med 36 1147 1936

Dail C W Affeldt J E and Collier C R Clinical aspects of glossopharyngeal breathing J A M A 158 445 1955

Dickinson D G Wilson J L and Graham B D Studies in respiratory insufficiency I Carbon dioxide and oxygen studies in early respiratory paralysis in poliomyelitis A M A Am J Dis Child 86 265 1953

Ferris B G Jr Meade J Whittenberger J L and Saxton G A Jr Pulmonary function in convalescent poliomyelitis patients III Compliance of the lungs and thorax New England J Med 247 390 1952

Ferris B G Jr Warren A and Beals C A The vital capacity as a measure of the spontaneous breathing ability in poliomyelitis New England J Med 252 618 1955

Landauer K S A national program of respiratory and rehabilitation centers (see pp 633 636)

Linderholm H and Werneman H On respiratory regulation in poliomyelitis convalescents Acta med scandinav (Supp 316) 154 135 1956

Lukas D S and Plum F Pulmonary function in patients convalescing from acute poliomyelitis with respiratory paralysis Am J Med 12 388 1952

Northwest Respirator and Rehabilitation Center and The King County Hospital Seattle Unpublished data

Plum F and Dunning M F Technics for minimizing trauma to the tracheobronchial tree after tracheotomy New England J Med 254 193 1956

Plum F and Swanson A G The development

of centrogenic respiratory failure in poliomyelitis Tr Am Neurol A p 131 1956
—— Abnormalities in the central regulation of respiration during acute and convalescent poliomyelitis A M A Arch Neurol & Psychiat (In press)
Radford E I Jr Ferris B G Jr and Kriete B C Clinical use of a nomogram to estimate proper ventilation during artificial respiration New England J Med 251 877 1954
Wilson J L and Dickinson D G Prevention of long time dependence of poliomyelitis patients on tank respirator J A M A 158 551 1955

lescence an endurance contest all patients were maintained on a program of progressively increasing their independent breathing times to tolerance Other therapeutic methods designed to strengthen the respiratory muscles were extremely disappointing Occasionally mastery of the technic of effective glossopharyngeal breathing was followed promptly by a rise in vital capacity of 30 to 50 per cent but this probably reflected increased chest compliance rather than improvement in strength of breathing muscles

It was our practice never to force to exhaustion those patients who claimed they needed assistance to sleep without respiratory aid no matter what the measurement of their vital capacity Gradually reducing respirator pressures or rocking bed during sleep helped a few patients in overcoming the need for nocturnal aid but it was usually more successful to wake the patient at 4 A M turn off the respiratory device at that time and urge him to return to sleep The dark and quiet ward the recent sleep and the knowledge of constant supervision reduced to a minimum stimuli which otherwise would contribute to arousal

Although our major emphasis in the paper has been on a program for respiratory emancipation it should be stressed that our goal in the rehabilitation of patients with respiratory insufficiency was more encompassing than the elimination of respirators Some patients never recover enough breathing function to become completely independent of respiratory aid Others were marginal and it was found that they were capable of much more active daytime programs if they were assisted at night with artificial respiration

Many of the patients who are referred to a respirator center are sent from other hospitals many months after illness because they have failed to become free of respiratory aid In many instances elimination of all use of the respirator has been set as the foremost goal of therapy Having failed to reach this goal these patients have suffered a deep sense of defeat and a long period of transition is required to redirect them toward potentially achievable objectives However once they accept the principle that a respirator is no different from any other crutch many of them can make remarkable strides Seventy patients have been discharged from our unit in the past 3 years 18 still require partial respiratory support but none require a tank respirator and most use a rocking bed only while sleeping Despite their partial dependence upon artificial respiration 12 of these 18 are actively engaged in either schooling housework or gainful employment All are home and all are active socially These are encouraging results and justify the care and effort required to develop a comprehensive program of respiratory emancipation and rehabilitation

BIBLIOGRAPHY

Affeldt J E Bower A G Dail C W and Arata N N The prognosis for respiratory recovery in severe poliomyelitis Arch Phys Med & Rehab 38 290 1957

Brahdy M B Late results of 63 cases of poliomyelitis treated in the respirator New York State J Med 36 1147 1936

Dail C W Affeldt J E and Collier C R Clinical aspects of glossopharyngeal breathing J A M A 158 445 1955

Dickinson D G Wilson J L and Graham B D Studies in respiratory insufficiency I Carbon dioxide and oxygen studies in early respiratory paralysis in poliomyelitis A M A Am J Dis Child 86 265 1955

Ferris B G Jr Meade J Whittenberger J L and Saxton G A Jr Pulmonary function in convalescent poliomyelitis patients III Compliance of the lungs and thorax New England J Med 247 390 1952

Ferris B G Jr Warren A and Beals C A The vital capacity as a measure of the spontaneous breathing ability in poliomyelitis New England J Med 252 618 1955

Landauer K S A national program of respiratory and rehabilitation centers (see pp 637 636)

Linderholm H and Werneman H On respiratory regulation in poliomyelitis convalescents Acta med scandinav (Supp 316) 154 135 1956

Lukas D S and Plum F Pulmonary function in patients convalescing from acute poliomyelitis with respiratory paralysis Am J Med 12 388 1952

Northwest Respirator and Rehabilitation Center and The King County Hospital Seattle Unpublished data

Plum F and Dunning M F Technics for minimizing trauma to the tracheobronchial tree after tracheotomy New England J Med 254 193 1956

Plum F and Swanson A G The development

of centrogenic respiratory failure in poliomyelitis Tr Am Neurol A p 131 1956
—— Abnormalities in the central regulation of respiration during acute and convalescent poliomyelitis A M A Arch Neurol & Psychiat (In press)
Radford E P Jr Ferris B C Jr and Kriete B C Clinical use of a nomogram to estimate proper ventilation during artificial respiration New England J Med 251 877 1954
Wilson J L and Dickinson D G Prevention of long term dependence of poliomyelitis patients on tank respirator J A M A 158 551 1955

DISCUSSION

DR AARSVOLD In weaning the patient from respiratory aid we have mainly followed the principles mentioned by Dr Plum However whereas Dr Plum s patients in the acute stage were mostly ventilated in the tank type respirators approximately one half of our patients were ventilated through tracheostoma by means of different positive pressure ventilators

This was due partly to the lack of a sufficient number of tank respirators and partly that we have received many of our patients from the provinces and until 2 years ago we did not have any portable tank respirators Now we have one but will still probably have to use positive pressure sometimes for transportation because of the difficulties in the communications in Norway

In contrast to the other Scandinavian countries we in Norway now prefer ventilation by means of tank type respirators when we have the facilities for it

We have used the pressure ventilators Aga Bang Lundia and Engstrom With each of them we have obtained sufficient ventilation However one of the main reasons for our preferring the tank type respirators to the pressure ventilators is that the latter to a greater degree needs trained and observant nurses In regard to the weaning from the respirator even those who are most enthusiastic about over pressure ventilators admit that emancipation from this type is much more difficult than weaning from a tank I have as yet no satisfactory explanation for this Perhaps the reason is a greater tendency to hyperventilate when we use a positive pressure respirator

A common experience to anyone working in the field of artificial respiration will be that the longer a patient is ventilated by means of one type of respirator the more difficult it will be to make him accept another type

Therefore the first step in weaning patients ventilated by a pressure respirator has been to shift them from one type of respirator to another Thus they realize that *it is possible* to breathe in different types of respirators

On e the acute stage has passed and the spontaneous ventilation has improved to the extent that the patient can use respiratory aid not giving as effective a ventilation then we try to use chest respirator and rocking bed We use the rocking bed as early as possible hoping to prevent perhaps two of the most common complications in respirator patients kidney stones and thrombophlebitis We have also tried to prevent kidney stones by giving enough fluid to keep the specific gravity of the urine below 1015 we have used salicylamide and we have used the standing bed as early as possible but nevertheless most of them have got kidney stones We have discussed the possibility of giving anticoagulants to prevent thrombophlebitis but have dropped it because of the danger of bleeding if a tracheotomy has to be performed

We have had the same experience as stated by Dr Plum namely that psychological factors can greatly influence the weaning of a patient Some years ago we considered it unthinkable to place several respirator patients in the same room and consequently each had his own Now we have learned how wrong this attitude was When the patients are together with other patients at the same stage of recovery this gives keen competition which can give astonishing speed to the process of weaning This is one of the reasons for treating patients in a respirator center instead of having them scattered about in the provinces with only a few patients in each hospital

Dr Plum mentioned that the patients who progressed the furthest were usually those who started in a comprehensive program of respiratory rehabilitation promptly at the end of their acute illness We have had similar experience but we have learned also that even those who start late may gain important improvement through effective therapy During the last years patients have been sent to us from the provinces where they have been ventilated by means of positive pressure ventilators for as long as 3 years without any improvement in the last 2 years However when they have been treated together with other patients and have made use of the equipment at the center to full extent

and of the experience of the personnel there we have seen definite improvement after a few months even in cases in which patients have been ill for as long as 3 years This has caused us to keep the patients at the respirator center for a considerably longer time than before Formerly we thought that after 1 year we could not obtain any further improvement but now we *know* that even after several years there can be important improvement through effective therapy

Another reason for keeping the patients in the center for a long time is that in the beginning for psychological reasons they are less co-operative Another example of the importance of the psychological factors is the striking improvement we often see when the patient has *visited home for the week end* Therefore we try as early as possible to get them home or at least outside the hospital for week ends and later on for longer leaves In that way we hope to avoid their getting tired of the treatment They need vacations now and then but according to our experience we do not discharge them finally before several years have passed

When the patients have learned frog breathing we are much more liberal in regard to sending them home for the week end We value frog breathing very much and therefore systematically try to teach it to the patients as early as possible Besides making the patients feel safe in knowing that they can manage without a respirator we regard frog breathing as being important in preventing reduced compliance Even with patients who have been artificially ventilated for several years we have seen improvement in ventilation capacity after they have learned frog breathing Glossopharyngeal breathing can partly substitute for an attachment on the tank for deep respiration Naturally we are careful not to let the patients exaggerate the frog breathing so as to give circulatory failure

We also regard frog breathing as being the best method for preventing respiratory complications during the weaning period If the patient is an effective frog breather we are not as strict as Dr Plum who demands 35 per cent of predicted vital capacity before decannulation We have removed the cannula on adults with a vital capacity of 300 cc and as yet we have not had to do a retracheotomy Even if all precautions are taken it is impossible to prevent infection in the respiratory tract now and then but by means of frog breathing tank with vacuum cleaner and postural drainage we have always succeeded in removing the secretion caused by common colds

We think it is important to remove the tracheal cannula as early as possible Even when the cannula is made of the least irritating material it will act as a foreign body in the trachea and will cause some secretion As long as the patient has the cannula there will be consequent secretions

We also try to avoid as early as possible suction by means of catheters inserted in the trachea because of the traumatizing effect of the catheter Therefore we teach the patients to get rid of the secretions by means of frog breathing or by tank respirator with vacuum cleaner and postural drainage We have not considered Teflon plugs as necessary

Like Dr Plum we do not think that the main thing is to get rid of respiratory aid We prefer the patients to be able to do work *with* a respirator instead of lying quietly in bed without respiratory equipment

Circulatory Disturbances in Life-Threatening Poliomyelitis

DR WILLIAM A SPENCER

I trust that my remarks will be received strictly as opinions Disturbances of actions of the heart and of the systemic and *pulmonary* circulation contribute to death processes in the polio patient with viral invasion of the brain stem or with conditions leading to an asphyctic state such as failure of breathing The conditions of grave concern in our experience include pulmonary edema hypotension vasomotor lability peripheral circulatory stasis and hyperthermia Irregularities of cardiac rhythmicity and myocardial insufficiency occur The complex nature of these conditions suggests that it would be useful first to consider practical experience with diagnosis and treatment of major circulatory abnormalities and then to make a very brief presentation of some of the patterns of disturbances observed in the patient

The experience to be described has been based upon cardiovascular disturbances observed in a regional respirator center which admits acute patients and has admitted over 1 200 since its opening Without consecutive clinical experience and physiologic investigation one might not be aware of the pitfalls of generalization We believe that we recognize some of these pitfalls and they are resultants of the very complicated behavior of the cardiovascular system in severe polio We think this behavior is a consequence of disturbances of the neural regulation of heart action and circulation biochemical disturbances of the extra and intracellular fluid compartments or mechanical malfunctions which arise in the course of the disease its complication or even of its treatment

Table 165 indicates the frequency of cardiovascular disturbances observed in what we call life threatening poliomyelities (after Dr Lassen) In 1 000 paralytic patients which we studied 357 were considered to be severely ill approximately 35 per cent The most common complication was respiratory muscle paralysis in 72 per cent of the severely ill Impairment of swallowing of a severe degree occurred in 60 per cent disturbances of respiratory regulation in 27 per cent and approximately 35 per cent had disturb*ances of circulation Now this group of 176* individuals who had circulatory disturbances of one or several types accounted for 52 of the 59 fatalities in the entire life threatened group including those admitted dead on admission for whom it would be impossible to categorize the cause of death It should be inferred that mor

TABLE 165 FREQUENCY OF CARDIOVASCULAR DISTURBANCES IN PATIENTS WITH LIFE THREATENING POLIOMYELITIS

CATEGORY	INCIDENCE	PERCENTAGE
Total severely ill patients	357	
Respiratory muscle paralysis	257	72
Impairment of swallowing	213	60
Disturbances of respiratory regulation	96	27
Disturbances of circulation	126	35

These ond tion e ot mutually ex l sin e com b n tion oc u in the sam nd v dual The physi l gi imp irment is ind c t d r th tha anatomi l cal zati n of the d ase p oce Nearly all the p ti nts co dered to h th eat to l fe had e ther resp atory muscle p alysis or impa rm nt of vall w ng or b th ond ti n F tte n nd du l ith respi to v rre l ts not requir g p t rs aid have been in luded becau of oe ten e of s allow ng mpa rment g of cepha litis or major c rcul tory d tu bance 55 ind l al are nclud d w th r p t ry n l paralys judg d n t to b f uffi t e e iry to req breath g aid but u u ll d tifi d in combination w th sw ll wi g im p ment n i ard ascular p obl s o h llv p sent s gn and symptoms of impendi g e e e dis ea e The o er all s r ts of the g o p i i l at d by the need of resp rat ry a l for periods i xce ot ? eeks in 80 of th ndi idu ls th re p t ry m sl paralysis Trache tom was nec ary in 0 th m pa red swall v ng becaus of way ob tru ti or the co b nat on of r p ratory muscle paralys equ ring arti fi al r p rat on and in 40 of th e th s vall i s d ffi ult es alone The tab l t n e l d p t nt v th bulbar poli myel t s ith isol te l p l tal m cle pa l s s faci l pa l si x l r pa l and in a f w i stan p r l t ngu paral r m set r i l m nt ho oth rs se d d not appe r to be se erely ll

TABLE 166 TYPE AND RELATIVE FREQUENCY OF CIRCULATORY DISTURBANCES OBSERVED IN 357 POLIOMYELITIS PATIENTS WITH THREAT TO LIFE

TYPE OF DISTURBANCE	INCIDENCE
Cardioregulatory disturbances and vasomotor lability	8
Hypertension of any degree	69
Myocardial insufficiency	58
Hypotension	50
Cutaneous vasoconstriction and hyperthermia greater than 105 F (rectal)	34
Gastro-intestinal bleeding hematemesis or melena	?
Pulmonary edema	11
Total number of individuals with one or more circulatory disturbances	126

These ncd e figu e t n u u ex u c mbi a n f the ond u n x u h m nd l a Th l l n h pe s m d gree (b sed on ta d d a u f k n pa a pol l w h se d se se n clud d n th tabul n b m l nc gul ry d u ban es u h a h a l b l d a ndu u d b n s d ab n h extrot l am n t o be insid l s sen f h o l e pe n n h l f n ho e c n n l a p pe t h d l h th ar te muscle p l r w w ng in m nd b n n n h d h l sev po ng a th e to lif

tality appears to go hand in hand with these disturbances of circulatory function This is simply a statistical association and not proof However it does corroborate our clinical relationship in which one sees the occurrence of these conditions at the time of rapid deterioration of the patient

Table 166 indicates the simple numerical incidence of the varieties in complications mentioned I did not construct percentage figures because of the extreme variability The most common condition simply numerically was cardioregulatory disturbances and vasomotor lability Hypertension of any degree not necessarily severe in the life threatened group occurred in only 69 individuals myocardial insufficiency in 58 hypotension in 50 and so on cutaneous vasoconstriction with hyperthermia up to 105 or higher greater than 105 in 34 individuals mass se gastro-intestinal bleeding in 27 and clinically evident pulmonary edema in 11 individuals

TABLE 167 TYPE OF DISTURBANCE AND ASSOCIATED MORTALITY IN 126 POLIOMYELITIS PATIENTS WITH CIRCULATORY ABNORMALITIES AND RESPIRATORY MUSCLE PARALYSIS OR SWALLOWING IMPAIRMENT

TYPE OF DISTURBANCE	ASSOCIATED % MORTALITY	INCIDENCE
Pulmonary edema	89	11
Hypotension	2	50
Vasoconstriction and hyperthermia	68	68
Gastro-intestinal bleeding	48	2
Myocardial insufficiency	36	58
Cardioregulatory disturbances	35	78
Hypertension	77	69
Total incidence of circulatory disturbances		3 7
Total number of individuals		126
Fatalities		52

F wo f h 126 n l u l (41 xp d wh h l b n l p n l a wa w g d ff ul b h nt f w l o e l n dm l h n a d n f la s u l be abl h d h l f he u a d l f n m f figu e O l n he 35 n l dua w h f h n g po (T b 165 h w 5 l w h n 45 f h nse f d

Now Table 166 should be construed to represent only the incidence in severe disease of the life threatening type defined before and not over all expectations with severe paralytic poliomyelitis

In Table 167 these simple numerical incidence figures have been rearranged according to the percentage of associated mortality in order to call attention to those conditions which require most urgent treatment or prevention Here one sees that the least common condition in our experience is pulmonary edema of which there were 11 examples and 89 per cent expired

Next in order of importance with respect to associated mortality was hypotension then peripheral skin vasoconstriction of severe degree associated with high body temperatures GI bleeding myocardial insufficiency cardioregulatory disturbances and last hypertension

Table 167 simply indicates the percentage of fatality with a particular complication regardless

of its frequency and should be taken to indicate only the likelihood of associated death and not the complication itself as the cause of death *which is quite often the result of many causes*

The development of cardioregulatory and vasomotor disturbances in association with lower brain stem involvement of the swallowing centers has been our general experience both clinically (Table 168) and as a result of neuropathologic examinations of the brain stem On the *other hand the occurrence of circulatory disturbances* with respiratory insufficiency in its terminal stages is also a matter of common clinical experience with the signs and symptoms of asphyxia One sees the same hypotension the same disturbances of cardiac regulation in both circumstances Separation of the events then of disturbance of cardiovascular function does not necessarily separate causes *from effects or from associated conditions* Certainly functional disturbance of the central nervous system can contribute to these conditions For example biochemical alterations of hypoxemia rapid shifts of intercellular and extracellular pH concentration carbon dioxide —all have been shown to disrupt neuronal *function So* once the central nervous system mechanism is disturbed either directly by invasion of the virus or indirectly by virtue of biochemical disturbances the end results may be quite similar Also it is true that these latter changes may be compounded with what we

TABLE 168 CARDIOVASCULAR ABNORMALITIES OF MAJOR SIGNIFICANCE IN ACUTE SEVERE POLIOMYELITIS

CONDITION	DIAGNOSTIC CRITERIA	% INCIDENCE*
Regulatory Disturbances		
Heart		
Sinus tachycardia	Peripheral pulse rates above those expected for temperature Severe tachycardia greater than 200 in children and 180 in adults Occurs in less than 5%	27
Sinus bradycardia	Sudden and transitory decrease in pulse rate to 80 beats per minute or less with fever Often associated with increasing temperature of the body and hyperthermia	17
Wandering auricular pacemaker	Changing PR interval in the ECG usually transitory or associated with conduction defects Not grave unless there is hypoxemia	7
Premature beats of ventricular origin	Premature complexes in a series may be of grave significance if there is diminished pulse volume or dropped beats	6
Supraventricular tachycardia	Paroxysmal rapid rates or may remain rapid and unmodifiable	1
Systemic circulation		
Hyperthermia	Temperature elevations in excess of 105 F (rectal) — 40 5 C Often coincides with severe generalized cutaneous vasoconstriction	24
Cutaneous vasoconstriction	Patchy purplish mottling of the skin with cutaneous cyanosis and sluggish circulation Skin temperature is cold Occurs just in the hands and feet with hyperthermia in children Crave if generalized	22

Incidence figures are relative and are based on the last 100 poliomyelitis patients admitted to the respirator center with a threat to life due to respiratory muscle paralysis or swallowing difficulty Mortality was 9 [illegible] in this group of patients 55 patients had swallowing difficulty 29 of these were combined with respiratory muscle paralysis the remainder had respiratory muscle paralysis alone 73 patients required prolonged respiratory aid.

TABLE 168 CARDIOVASCULAR ABNORMALITIES OF MAJOR SIGNIFICANCE IN ACUTE SEVERE POLIOMYELITIS (*Continued*)

CONDITION	DIAGNOSTIC CRITERIA	% INCIDENCE
Systolic and diastolic hypotension	Systolic pressures less than 90 mm in the febrile adult and 60 mm in the child. Often associated with mortality hypoxemia terminal asphyxia or artificial respiration in the presence of vasomotor incompetence	15
Systolic and diastolic hypertension	Systolic pressure greater than 200 mm of mercury in adults and 160 mm of mercury in children. Lesser degrees are self limited unless indicating progressive elevation. Severe degrees can precede pulmonary edema and congestive failure	10
Pulmonary circulation Pulmonary congestion	A condition of increased bronchovascular markings in the x ray. Decreased distensibility of the lungs during artificial respiration. ECG indicates high peaked P waves right axis P vector rotation and clockwise rotation of the QRS vector	12
Pulmonary edema	Acute respiratory distress with moist crepitant râles in the lungs. Bronchiolar constriction may be present especially if hypoxemia develops. Pink frothy secretions fill the trachea bronchial tree. May occur with congestive heart failure or following severe hypertension	7
Myocardial Disturbances Myocardial insufficiency	Poor soft quality of the heart sounds. Rapid heart rate. Gallop rhythm may be present. Cardiac enlargement may or may not be observed. ECG indicates separation of the QRS and T vectors, ST segment depression, prolongation of QT time, T wave shape changes and low voltage. Best indicated by serial changes in successive ECG s. ECG and heart sound registration show marked shifts in the duration of mechanical systole (onset QRS to beginning of second heart sound). Can be secondary to pulmonary complications, hypoxemia, electrolyte alterations and myocarditis	17
Autonomic Disturbances Gastro intestinal bleeding	Often occurs with ileus and gastric atony. May follow episodes of severe bradycardia. Blood tinged or chocolate gastric secretions. Melena	20

would like to call regulatory or compensatory adjustments to the actual disturbances taking place in the circulatory system. For example if the blood pressure falls this calls into play immediately from the carotid sinus mechanism reflexes which tend to elevate blood pressure toward its normal value. When this circumstance occurs at a time when the central nervous system apparatus is damaged you can see how complicated the interpretation or the results may be. Clinical investigation and experience may tend to substantiate one or another cause of circulatory disturbances as prepotent in any individual patient or at a particular time. It is probable that processes of the disease physiologic effects of the treatment procedures and the aggregation of complications have a variable timetable.

Figure 374 shows one clinical example which reveals the chaotic disturbance of circulation with which you are all quite familiar. This was a 28 year-old man who had total muscular paralysis swallowing impairment and required artificial respiration. In the upper half of the chart the solid black dots connected by a continuous line are a record of the systolic blood pressure which proceeded from high levels of 190 200 mm of mercury and over a course of some hours gradually declined to low levels and then spontaneously recovered until terminally when the blood pressure suddenly dropped and the patient expired in a matter of 8 minutes

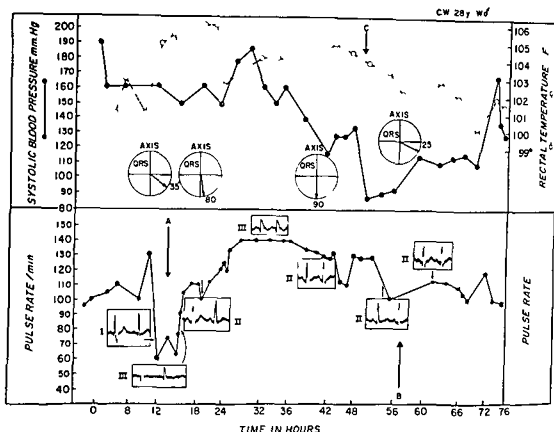


FIG 324 Bradycardia hypertension hypotension cardiac conduction defects and hyperthermia in bulbospinal poliomyelitis with neuropathologic evidence of medullary involvement Serial pulse rate blood pressure body temperature and representative serial electrocardiograms are oriented in relation to time from admission At point A Banthine was administered at point B ephedrine sulfate at point C intravenous amino acid solution The chronology of the major cardiovascular disturbances is indicated in a patient with swallowing difficulty respiratory muscle paralysis and quadriplegia

The hollow circles with the dash lines indicate the body temperature (rectal) On the electro cardiogram a QRS deflection is indicated in the circles below and shows considerable degree of vector shift during the course of hours Below we see the pulse rate remarkably variable with an episode of bradycardia (pulse rate of 60) at a time when the body temperature was 106 and associated with that representative electrocardiograms which show changing conduction patterns beat by beat in the electrocardiogram For example there is intermittent right bundle branch ventricular block appearing and dis appearing Now this has been described in normal individuals frightened by unexpected gunshot so we suspect that such changing pat terns may be on the basis of neuroregulatory disturbances rather than disturbance of the heart itself Finally there is a persistent right bundle branch block In this patient after his unfortunate demise a careful evaluation of the brain stem revealed extensive involvement in the medial reticular formation nucleus of the vagus the nucleus ambiguus field H of Forel and many other areas of the brain stem which are considered to be part of the hierarchy of central nervous system cardiovascular regulation

Figure 325 indicates the development of hypotension and bradycardia as a result of transient arterial hypoxemia in a patient requiring con

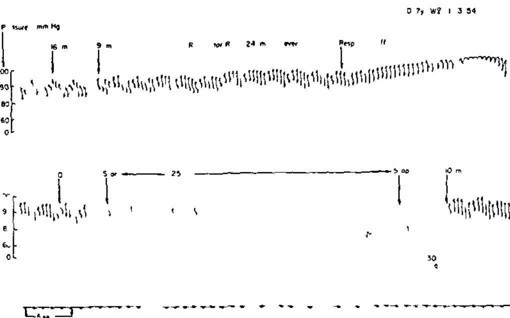


FIG 375 The genesis of bradycardia hypotension and cardioregulatory disturbances by hypoxemia in bulbospinal poliomyelitis Percentage saturation of femoral arterial blood is indicated on the right of the figure and pulse rate values on the left Representative ECG s A B and C coincide with 40 60 and 80 minute time intervals Equivalent ventilation volumes in both tank and cuirass respirators were assured by spirometry The patient was confined to the tank respirator except for the period indicated cuirass Blood pressure values are indicated along the abscissa

stant artificial respiration due to respiratory muscle paralysis and severe swallowing difficulty This individual to be sure had evidence of severe brain stem invasion with his swallowing difficulty but had a fairly stable heart and blood pressure picture until the time that we introduced for a brief period a cuirass respirator for nursing care In this chart on the vertical dimension the pulse rate is indicated by the hollow circle connected by broken lines The arterial oxygen saturation of the femoral blood by direct sampling not by oximeter is indicated by the solid black circle connected with an uninterrupted line Above are represented electrocardiograms in the boxes marked A B and C Blood pressure is indicated here in the control period at the time of hypoxemia and following hypoxemia The patient was changed from the tank respirator where he had essentially normal arterial oxygen saturation and a pulse rate of 120 to 130 and then into a cuirass respirator for a period of about 8 minutes Arterial oxygen saturation fell to 60 per cent although the level of ventilation remained the same Associated with this was a decline of pulse rate from 130 to approximately 80 beats per minute The ECG indicated the manner of cardiac slowing and the ECG components following the hypoxemic episode did not return to the previous configuration After prompt return to the respirator and the temporary use of high oxygen concentration his former status was not quite resumed Now we have a patient who has undoubted evidence of brain stem involvement but who was reasonably stable until accidentally subjected to hypoxemia and it unveils disturbances of regulation of these vital functions that we have just seen

Figure 376 is a continuous femoral arterial pulse recording from a 7 year-old child who had swallowing difficulty She had sufficient right diaphragm and intercostal paralysis requiring

artificial respiration for a period of 4 weeks On the surface I noticed that this patient had marked lability of skin color varied from blanching to suffusion and that the blood pressure seemed to vary obviously with the cycling of the respirator which of course is not unfamiliar to many in this audience On the vertical dimension of the chart the calibration of the blood pressure is in mm of mercury The tank respirator cycling was at minus 16 cm Pay attention only to mean blood pressure because we do not feel this recording is accurate enough to interpret pulse pressure Mean blood pressure tends to be around 90 minus 16 It goes higher when the respirator pressure is decreased to minus 9 and then when the respirator is turned off the blood pressure goes on off the record although it tends to come down immediately In the lower record we increase the negative respirator pressure to minus 25 and you see a decrease in mean blood pressure which is quite significant in approximately 30 seconds Now the point here was that this patient required careful minimal respirator pressures at higher rates to minimize the circulatory effect of pressure breathing probably coming about because of vasomotor incompetence and inability of the person in this circumstance of polio to adjust venous return to the thorax in the condition of pressure breathing Now briefly the conditions in order of importance that one would diagnose are pulmonary congestion which is a condition of increase bronchovascular markings in the roentgenogram decreased distensibility of the lungs ECG changes Pulmonary edema by our clinical definition is occurrence of pink frothy secretion in the tracheobronchial airway with acute respiratory distress The next most im

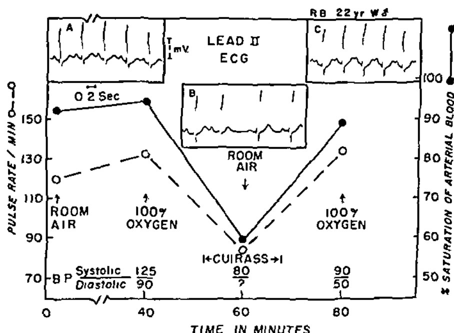


Fig 326 The effect of tank respirator pressure breathing on the femoral arterial blood pressure in a poliomyelitis patient with vasomotor incompetence Sections of directly recorded femoral arterial blood pressure are indicated in relation to tank respirator pressures and the cessation of artificial respiration The blood pressure values are shown in relation to pressure calibration in mm of mercury on the ordinate at the left of the scale Time calibrations are along the abscissa of the record and apply except where gaps are indicated Cyclic variations are due in part to the placement of the pressure transducer outside of the chamber of the respirator Pulse pressure fluctuations are to be considered only representative and not true values because of the small caliber of the arterial needle

portant condition we mentioned was that of hypotension which we recognize as systolic pressures in the adult of less than 90 mm of mercury in the state of fever and 60 mm of mercury less than 60 in the child Cutaneous vasoconstriction is self-evident However it is misleading We believe that it in fact contributes to decrease heat loss from the skin and that is why it is so commonly associated with elevation of body temperature The regulatory disturbances are self-evident. Myocardial disturbances I would like to discuss briefly Disturbances of cardiac activity in the course of polio are being recognized with increased frequency This undoubtedly reflects electrodiagnosis in appreciation of lesser degrees of myocardial insufficiency rather than frank congestive heart failure The most useful measures for us have been serial pulse and blood pressure records of the type appreciated by the anesthesiologist Careful serial electrocardiograms and simultaneous phonocardiograms for measurement estimation of systolic duration routine fluoroscopy and occasionally special studies of the type shown in Table 169 are recommended

TABLE 169 INCIDENCE OF CHANGES IN SERIAL ELECTROCARDIOGRAPHIC AND PHONOCARDIOGRAPHIC MEASUREMENTS IN ACUTE POLIOMYELITIS WITH THREAT TO LIFE

MEASUREMENT AND CONDITION	% INCIDENCE
SCALAR	
PR interval prolongation	6
QRS interval prolongation due to bundle branch block	9
QT interval prolongation	28
QT interval shortening	7
T wave flattening	5
U wave deflection	17
VECTOR (D rection and magnitude)	
Right atrial preponderance	15
Left atrial preponderance	6
Right ventricular preponderance	22
Left ventricular preponderance	3
SYSTOLIC DURATION (Relative to hea t rate)	
Systolic prolongation	11
Systolic shortening	41

Th se figu ar ba ed o the lysi of s r al elect a d gr m and ph ocard g m the l st 100 t pol my l u p u ts w th th eat to l f

Now the ECG is important to us because in a careful study of 350 individuals shown in Table 169 approximately one fourth show QT interval prolongation 17 per cent U wave deflection about one fourth right ventricular preponderance in the vector analysis and 15 per cent right atrial preponderance Nearly half of the individuals showed marked shortening of systolic duration corrected for the rate of the heart In about 10 per cent systolic prolongation occurred

In the present state of our knowledge it is obviously difficult to correlate these different changes with pathologically demonstrable lesions in the myocardia or conduction system However that the changes have an extreme chronologic variation in the same individual suggests the possibility that they are related to current dynamic changes of strength of contraction ischemia or state of nutrition of the muscle metabolic disturbances of the cells and indeed even altered neural regulations

Satisfactory treatment can be successfuly carried out in some individuals (Table 170) Survival in the presen e of usually lethal complications must be attributed to many factors and the relationship of favorable results to specific treatment viewed with considerable caution However the declining incidence of circulatory catastrophe may be cited It has paralleled earlier use of artificial respiration better management of airway problems improvements in respiratory equipment and its usage employment of physiologic measurements to estimate ventilation the avoidance of overtreatment Furthermore I thing the prompt correction of hypotension with such drugs as Levophed administered with great care the use with proper indication of digitalis derivatives the use of Rauwolfia serpentina extracts at appropriate times careful fluid and electrolyte management the avoidance of overloading of the circulation the prompt correction of pulmonary complications and many other things put together appear to have some benefit in these dreadfully complicated circumstances I do not have time to go into detail in our particular plan of treatment but I would be delighted to see any one of you at our exhibit and discuss with you the management of these conditions which as I have inferred are quite complicated I would like to say in conclusion that

TABLE 170 TREATMENT OF CARDIOVASCULAR ABNORMALITIES IN ACUTE POLIOMYELITIS

CONDITION	TREATMENT	DOSAGE
Sinus tachycardia	Usually no therapy used unless extreme (See text)	
Sinus bradycardia Autonomic disturbances such as gastro intestinal bleeding	If persistent use vagal blocking agents Methantheline bromide Oral medication indicated through nasogastric tube if gastro intestinal bleeding is present Also use con stant nasogastric suction Atropine is con traindicated Transfusion for blood loss	Banthine® 5-10 mg adminis tered parenterally Oral dose 25-50 mg every 6 hours for 24 to 72 hours Amount depends upon hemoglobin and hematocrit.
Disturbances of cardiac rhythm	Usually no treatment unless hypoxemia is the cause Oxygen therapy with adequate *pulmonary ventilation*	
Hypertension	Indicated if systolic pressure exceeds 180 mm of mercury in the adult and 150 mm of mercury in the child Extracts of Rauwolfia serpentina for parental use Hydralazine and hexamethonium derivatives contrain dicated	Rescinnamine® 160 Micro grams /kg of body weight /24 hours One fourth calculated amount given immediately in travenously balance in di ided doses every 6-8 hours
Hypotension	*Drug therapy indicated if systolic pressure* falls to 80 mm of mercury Norepinephrine Other vasopressors less effective or incon tant	Levophed® 4-8 mg per 1 000 ml of intravenous solutions Rate of administration depends upon pressor response
Myocardial insufficiency	Pressure adjustments of the pressure breath ing device in respiratory patients See text for important general considera tions and indications for drug therapy	See text for technic of adjust ment of respirators
	Digitalis derivatives Administer cautiously hypersensitivity to effect may be present	Digitoxin® 025 to 010 mg total dose /kg of body weight in *one* 24 hour period Admin ister one fourth immediately Control succeeding amounts by electrocardiographic evaluation (See text) *Balance in 6-8 hour* intervals in divided doses
	Rauwolfia erpentina derivatives	Rescinnamine® Parenteral dos age same as treatment of hyper tension First parenteral dose may be followed by oral admin istration of 0 25 mg of Ser pasil® every 6 hours
Hyperthermia	Treatment indicated for body temperature in excess of 104 F (rectal) alternate appli cation *of warm and cool sheets to the body* with brushing of the skin to stimulate circu lation Usually needed if hypertension is *coexistent* Alcohol may be used intravenously Acetylsalicylic acid not indicated Avoid al cohol rubs and ice packs unless hypothermia is desired	Alcohol 5% solutions in 5% and 10% dextrose or invert sugar in water 500-1000 ml may be given intravenously depending upon age

TABLE 170 TREATMENT OF CARDIOVASCULAR ABNORMALITIES IN ACUTE POLIOMYELITIS (*Continued*)

CONDITION	TREATMENT	DOSAGE
Acute pulmonary edema	Establish adequate pulmonary ventilation with intermittent positive pressure breathing apparatus and administer high concentrations of oxygen Drug therapy may be helpful Rotation of tourniquets on the extremities If treatment is unsatisfactory nebulization of aerosols may be tried Electrophrenic respiration if available Treat myocardial failure if present	Aminophylline 1 to 5 Gm administered slowly intravenously with 5 to 50 ml of 50% glucose

Severity, age of the patient, persistence of the complication and adequacy of the response determine the duration of treatment

the pathologic physiology occurring in poliomyelitis complicated by respiratory insufficiency or swallowing difficulty is variable Careful and constant individualization in the planning and execution of remedial preventive measures is of extreme importance The timing and use of such measures or indeed the withholding of treatment are clearly matters of judgment experience and investigation of abnormal functions

It is hoped that these "experiments of nature in pathologic physiology will no longer result from the invasion of the nervous system by the poliomyelitis virus The advent of a successful vaccine widely employed clearly ensures this eventuality It is hoped then that descriptions of the chaos brought by this infection can then be relegated to obscurity except for those principles that are axiomatic and apply to any catastrophic illness posing a threat to life through respiratory and circulatory dysfunction

Biochemical Changes in Poliomyelitis Patients

DR INGEMAR JUNGNER

The first large scale biochemical investigations which serve to establish the premises for rational care of poliomyelitis patients were made by Bower *et al*[1 5] in connection with the wide spread epidemic in Los Angeles in 1948 Their comprehensive studies disclosed severe biochemical changes particularly with respect to the acid base and electrolyte balance and nitrogen metabolism The experience of Bower *et al* was subsequently put to good use in the large epidemics which broke out in Scandinavia—in Copenhagen in 1952[6 8] and in Stockholm in 1953[9 10] This also applied to the considerable methodologic and technical improvements introduced by Astrup *et al*[11 13] for the laboratory control of patients with disturbances in the acid base balance

As far as the *acid base balance* is concerned the usual disturbances are due to the altered gas exchange The acidosis and alkalosis which then arise are thus *respiratorily* conditioned and are to be ascribed primarily to alterations in the CO tension (pCO) of the blood The organism has only a limited defense against these changes in the degree of acidity Therefore it attempts to maintain a constant pH in the blood — i e to normalize the ratio of bicarbonate to CO in Henderson Hasselbalch s equation—by a compensatory adaptation of the blood content of bicarbonate Consequently exact determinations of the *CO tension are a prerequisite for therapeutic* measures in cases of respiratory insufficiency This presupposes adequate laboratory resources irrespective of whether the tension is determined directly or is calculated indirectly from exact determinations of the pH and bicarbonate content

In the usual conditions of metabolic acidosis or *alkalosis* the plasma bicarbonate is on the contrary th first to undergo a change compensatory normalization of the pH being brought about by a change in the CO tension Consequently with a metabolic disturbance alone it is often possible in practice to *rely only* on determinations of the plasma bicarbonate measured as total CO actual bicarbonate CO_2 combining power or standard bicarbonate (Table 171)

The conditions become still more complicated if *both* respiratory and metabolic disturbances are present[14 16] This is not uncommon in poliomyelitis although the incidence seems to vary appreciably in different epidemics For example in the 1952 epidemic in Copenhagen a relatively large number of patients had a marked rise in nonprotein nitrogen and an element of renal acidosis[6] In the Stockholm epidemic in the following year we had on the contrary a very low incidence of such complications[9] and the changes were marked in only a few cases (Fig 327) However the incidence of metabolic acidosis has been considerable in later sporadic

TABLE 171 PLASMA BICARBONATE

SYMBOL	UNIT	DEFINITION
Total CO	mMol/l	Total content of CO_2 in blood or plasma estimated after expulsion through addition of acid
Actual bicarbonate	mMol/L	Bicarbonate content of plasma at actual pCO
CO Combining power	mMol/l	Bicarbonate content of plasma separated at the *actual* pCO of blood and *then* equilibrated at $pCO_2 = 40$ mm Hg
Standard bicarbonate	mMol/L	Bicarbonate content of plasma separated from fully oxygenated blood at 38 C and $pCO_2 = 40$ mm Hg

According to Astrup[13]

cases of poliomyelitis Therefore the possibility of a metabolic element in poliomyelitis should be borne in mind and the chemical analysis aimed at finding out whether or not a metabolic disturbance is present and if so its degree

The difficulties of evaluating the acid base balance in cases of poliomyelitis with respiratory paralysis are associated with the fact that the compensatory mechanism of the body cause different conditions to present great similarities Moreover the relation between pH CO tension and plasma bicarbonate is a logarithmic one A feature of practical importance from the therapeutic point of view is that the respiratory shifts in CO tension take place far more rapidly than the compensatory change in the bicarbonate content of the blood Therefore the conditions are simpler in the acute stage of poliomyelitis *and can be clearly presented in the form sug*gested by Astrup[1] He utilized the fact that the logarithm of the CO tension shows a linear relation to the blood pH at a certain bicarbonate content This implies that the primary respiratory changes are independent of the absolute bicarbonate content of a given plasma sample The metabolic changes in acid base balance as mentioned primarily cause changes in this bicarbonate content This offers a possibility to distinguish between the respiratory and metabolic disturbances when they occur simultaneously For if the bicarbonate is determined under standardized conditions with respect to temperature CO tension and also the oxygen saturation the secondary respiratory changes are eliminated A measure of the metabolic component alone is then obtained We have used Astrup's *standard bicarbonate* defined as the concentration of bicarbonate ion in the plasma

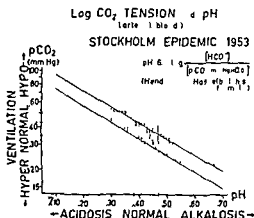


Fig 3?7 The appearance of the values obtained in the Stockholm epidemic when the logarithm of the CO tension is given as a function of the pH As mentioned the logarithm of the CO tension shows a linear relation to the blood pH and it can be inferred from this figure that the region for normal respiratory changes is relatively small and that there is a wide scattering of the values On the whole they nevertheless fall within the region where purely respiratory disturbances in the acid base balance are found or in its immediate vicinity Definitely divergent values i e cases with metabolic imbalance are few and some of them have a normal pH as an indication of compensation

Table 1/2 The Content of Total-CO Actual Bicarbonate CO Combining Power and Standard Bicarbonate in Plasma from the Same Blood Sample at Varying CO Tension and Oxygen Saturation Expressed as mMol/L[11]

Analysis	pCO 20 mm Hg	pCO 40 mm Hg	pCO 80 mm Hg	Sample
Total CO	16 8	2? 2	30 0	
Actual bicarbonate	16 2	?1 0	27 6	Fully
CO Combining power	18 0	2? 2	?7 5	oxygenated
Standard bicarbonate	21 0	?1 0	?1 0	blood
Total CO	19 6	25 7	34 8	
Actual bicarbonate	19 0	24 5	3? 4	Fully
CO_2 Combining power	21 0	25 7	31 9	reduced
Standard bicarbonate	?1 0	21 0	?1 0	blood

part of fully oxygenated blood at 38 C when the CO_2 tension is 40 mm Hg as a gauge in this respect

As seen in Table 172 the standard bicarbonate is constant at different CO_2 tensions in opposition to the total CO_2 the actual bicarbonate and the CO_2 combining power Moreover it is seen that according to definition the standard bicarbonate is equal to the actual bicarbonate content at a pCO_2 of 40 mm Hg The values are taken from a recent work of Astrup where he points out that the oxygen saturation affects the plasma bicarbonate That is why the plasma has to be separated at a *fixed* oxygen saturation and suitably separated from fully oxygenated blood

We have found Astrup s standard bicarbonate to be a reliable and probably the most convenient measure of the nonrespiratory components of the acid base metabolism in the organism

In Table 173 the different forms of disturbances in acid base balance are summarized The *respiratory* acidosis and alkalosis are recognized by changes in the *CO_2 tension* while the *metabolic* acidosis and alkalosis affects the *standard bicarbonate* The degree of metabolic imbalance *is obtained as the difference between the standard* bicarbonate calculated and its normal value and is shown in the last column This difference is *negative* in metabolic acidosis and *positive* in metabolic alkalosis

The metabolic component is of course especially important if the polio cases are complicated by for example diabetes However I would like to emphasize that an irrelevant factor also can influence these analyses which should be decisive for treatment with artificial respiration Table 173 shows a case which was not complicated by any organic disease but in which a mental depression brought about hunger acidosis reflected as moderate but fully evident metabolic acidosis

The necessary data for determining the patient s acid base balance on a given occasion thus are the pH the CO_2 tension and the standard bicarbonate Several methods can be used for the analysis However Astrup[1] has suggested a simple method for facilitating the analysis This consists of first determining the actual pH of the blood followed by a new pH determination at 38° C and a fixed CO_2 tension suitably 40 mm Hg, either on separated plasma or on fully oxygenated blood [13] Both the actual CO_2 tension and the standard bicarbonate can then be obtained from diagrams We have used this method of Astrup s as well as determinations of *the blood pH and total CO_2* and found the figures to be in good agreement

Disturbances in the *electrolyte balance* in poliomyelitis are due partly to the changes resulting from the altered gas exchange and partly to the

TABLE 173 EXAMPLES OF DIFFERENT FORMS OF DISTURBANCES IN ACID-BASE BALANCE

TYPE OF DISTURBANCE	pH	TOTAL CO_2 mMOL/L	pCO_2 mm Hg	STANDARD BICARBONATE mMOL/L	ΔHCO_3^{*} mMOL/L
Normal values (arterial blood)	7 35–7 45	20–29	35–43	21 7	±2 8 (28)
Respiratory acidosis	7 06	39 5	133	22 9	+ 1 2
Respiratory alkalosis	7 64	15 7	15	21 6	− 0 1
Metabolic acidosis	7 10	5 8	18	6 9	−14 8
Metabolic alkalosis	7 57	38 5	43	36 3	+14 6
Polio patient with occasional hunger acidosis					
Feb 2	7 36	20 8	37	20 2	− 1 5
Feb 3 A M	7 27	21 8	47	19 3	− 2 4
Feb 3 P M	7 18	16 8	44	14 9	− 6 8
Feb 4	7 21	19 6	48	17 0	− 4 7
Feb 8	7 37	25 3	44	23 4	+ 1 7

* *Difference in* standard bicarbonate = standard bicarbonate —21 7

losses through for instance secretions and sweating Finally they are a result of the raised urinary excretion of potassium and phosphate in particular associated with the excessive destruction of muscle

Of the changes resulting from impaired ventilation those in respiratory acidosis are the most important. They are associated with an increased urinary output of chlorides as a result of the increase in plasma bicarbonate However the changes in the chloride content of the plasma are generally so small that subnormal normal values are found Even if these respiratorily conditioned changes are not so marked it is important to take therapeutic measures for their compensation This is because they are greatly accentuated by considerable chloride loss through such paths as profuse sweating large quantities of secretion from the respiratory tract and gastric atony

The urinary excretion of chlorides is not accompanied by any sodium loss but by some retention in the body probably due to passage of sodium into the intracellular fluid Thus the organism as a whole does not suffer from sodium deficiency but the low values found in both blood and urine are largely to be ascribed to a pathologic distribution of sodium in the body Consequently an attempt to produce normalization by compensatory therapy may lead instead to an accentuation of this pathologic distribution Laragh[17] has shown that oral administration of potassium chloride to patients both with and without edema—although not with poliomyelitis—produces a rise in the blood content of sodium This seems to be of interest in poliomyelitis as well since it is hoped by the administration of potassium to exchange this substance for the increased intracellular content of sodium *i e* to produce the normalization implied by adequate therapy However the exact distribution of potassium and sodium between the intra and extracellular fluid has not as yet been sufficiently elucidated to permit any definite conclusions

It is usual in poliomyelitis to find—apart from a passing initial rise—definite fall in serum potassium and a rise in its urinary excretion[4 1 1] The muscle destruction releases large quantities of potassium but there is nevertheless a fall in its serum concentration Potassium deficiency *is generally regarded as being of such* importance that extra potassium is administered routinely Despite this it often may be difficult to normalize the blood values completely As Schribner *et al* have stressed it is necessary in the dosage of potassium to recall that through its influence on sodium and thereby on the chloride and bicarbonate content of the blood it will have an acidotic effect Therefore it will increase any respiratory acidosis and even cause symptoms of intoxication Consequently the potassium dosage must be individualized on the basis of the results of the chemical analysis taking into account such matters as the state of ventilation the extent of paralysis and the risk of renal complications

True overwhelming evidence exists in favor of the increased urinary output of K as the factor responsible for the fall in serum K But the possibility cannot be disregarded of a retention of K in the muscles as in familial periodic paralysis[3] for example However in the latter condition the potassium is usually protein bound to a considerably greater degree than normally and is not ultradialyzable Our analyses have not disclosed any change in the binding of serum potassium in poliomyelitis patients [4] Nor have ECG studies indicated any marked degree of potassium retention in the heart muscle at any rate

In poliomyelitis the electrolyte disturbances are somewhat difficult to compensate since the therapeutic range is narrow The risk of both under and overdosage is great and individualized therapy based on repeated chemical analysis is essential Experience indicates that the urinary excretion of chlorides should be kept on a fairly low level—below 60 mEq—/24 hours—and that Na should be administered restrictively and only together with K With respect to potassium we have usually given KCl in doses of 40 80 mEq—/24 hours Despite this dose or *even a higher one no less than 32 per cent of* the respirator cases in the Stockholm epidemic still had hypokalemia[9]

Fluid must be given to compensate for the often very large loss The risk of edema must not be underestimated since autopsy has disclosed (in addition to myocarditic and other changes) subclinical signs of pulmonary edema[3 6] even in cases where the analyses have indicated an adequate fluid balance When *large quantities of fluid must be given in the*

acute stage it is essential for blood plasma or hypertonic serum albumin solution to be given concurrently by the parenteral route[3,10] Administration of protein hydrolysates has been recommended[4] but administration of individual amino acids seems to be unfavorable judging by animal experiments[7]

In lengthy immobilization it is important even in the later stages to maintain a high diuresis In poliomyelitis as in many other immobilizing illnesses e g skeletal tuberculosis the urinary output of calcium is raised[8b,47] However the blood values are usually normal owing to mobilization of Ca from the skeleton The phosphate excretion is also raised and the high incidence of urinary concretions in the late stages of poliomyelitis is presumably due to the solubility product of Ca and phosphate being exceeded[9] The favorable effect of forced diuresis in such cases as in nephrocalcinosis is largely to be ascribed to the fact that it has been maintained throughout the 24 hours[30] A reduction in the urinary excretion of Ca P and N is also reported with steroid therapy[8c,31]

Poliomyelitis is associated with a great disturbance in *nitrogen metabolism* Owing to fever and inanition in connection with the onset and particularly the excessive muscle destruction due to paralysis the nitrogen balance is markedly negative A striking feature is that muscle destruction has a far more rapid onset and is more severe in poliomyelitis than in paralysis resulting from division of a nerve or a disease of the central nervous system for instance The *greatly increased urinary excretion of nitrogen* may amount to a total 25 45 g/24 hours[4,9] The maximum excretion is noted 5 16 days after the onset of paralysis The increase in total nitrogen excretion is represented chiefly by an increase in urea nitrogen whereas creatine nitrogen and *creatinine nitrogen play lesser roles* quantitatively as do uric acid nitrogen and amino acid nitrogen The amino acids have been studied but neither their quantity nor distribution has proved to be characteristic in poliomyelitis[8]

The fa t that urea nitrogen is *chiefly* responsible for the increased urinary output of nitrogen indicates that the breakdown of muscle protein largely follows the normal pattern and is practically complete Such rapid and complete breakdown must place great stress on the liver which is forced to deal with a synthesis of urea for instance which greatly exceeds the normal function Liver damage in poliomyelitis has been reported by several authors[8] even in the form of decreased serum cholinesterase a tivity with no other clinical signs of liver damage[33] In this connection it may be mentioned that we have determined the activities of glutamic oxalacetic transaminase malic dehydrogenase and lactic acid dehydrogenase in serum without finding any marked elevation[4]

Creatinuria is a constant feature in all severe cases of poliomyelitis but is also normally seen in connection with immobilization[34] In our experience the creatinuria in poliomyelitis has a surprisingly lengthy duration—several years—in relation to the relatively short period of very high urea excretion Several authors have found a relation between creatine excretion and muscle reduction but Hoagland Gilder and Shank[35] have pointed out that the decreased output of creatinine is a better measure of the reduction of muscle mass in progressive muscular dystrophy In our experience this also applies to poliomyelitis Under normal conditions the creatinine excretion is strikingly constant as compared with the large variations shown by other urinary components under the influence of su h factors as fasting consumption of meat and muscular exercise The creatinine coefficient *i e* the excretion in mg /kg of body weight per 24 hours is given as about 23 under normal conditions We have found this coefficient to be *de reased in poliomyelitis* in one of our cases it was as low as 7 mg /kg /24 hours A coefficient in relation to the muscle mass alone—and verified for poliomyelitis patients—would be of great value as an objective gauge of the extent of paralysis Also it would be of interest to *analyze the enzymes in the muscles regulating* the metabolism and function of creatine In poliomyelitis[4] as in muscular dystrophy[38] there is a disturbance in creatine metabolism so that a large proportion of an oral dose of 1 to 3 grams is recovered in the urine whereas normally the whole amount is utilized

Provided that renal function is unimpaired the values for the blood content of urea a d creatinine show little variation To find out the way in which the muscle breakdown products

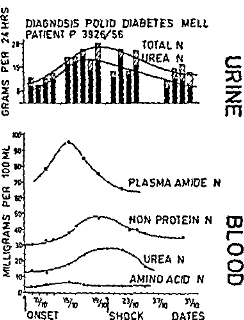


Fig 328 Case of poliomyelitis with diabetes mellitus

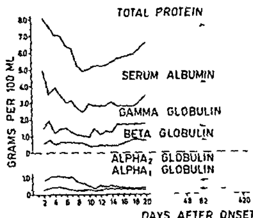


Fig 329 Serum proteins in a case of severe poliomyelitis (paper electrophoresis Veronal buffer pH 8 6)

reach the liver Astrup *et al*[8] analyzed the amino acid content of the blood but found no rise We have analyzed the various nitrogen fractions in the blood of poliomyelitis patients but were also unable to find any change in the amino acid content Nor did we detect any signs of degraded muscle protein or sufficient quantities of unaltered myoglobin to explain the considerable nitrogen transport On the other hand the amide nitrogen fraction of protein exhibited such a large rise during the acute stage of severe poliomyelitis that it could a count for the nitrogen transport [1]

Figure 328 shows a cas of poliomyelitis with diabetes mellitus In the a ute stage there is a considerable increase of total nitrogen and urea excretion in the urine Of different nitrogen fractions investigated in the blood the plasma amide nitrogen shows an increase in the early stag Later in connection with a diabetic coma and shock there was also an increase of the nonprotein nitrogen and the urea nitrogen The amino acid nitrogen is within normal limits throughout the muscle destruction associated with poliomyelitis

The negative nitrogen balance is reflected as a decrease in serum albumin [3] The decrease is in proportion to the nitrogen deficit and thus to muscle destruction and is a constant finding in se ere ases The total serum protein decreases but the greatest variation occurs in the albumin fra tion This starts to fall soon after the onset and reaches a minimum during the 5th to the 10th day in severely paralyzed cases it even falls below the edema limit (about 3 g 100 ml) We have made paper electropho etic analysis in a large number of cases We then found no significant changes in the other serum fractions apart from a moderate rise in the gamma globulin fraction 2 or 3 weeks after the onset and an irregular increa e of the alpha globulin (Fig 329)

The decrease in serum albumin has been presumed to depend on impairment of liver function but it should probably be seen also against the background of ery rapid metabolism Like liver protein serum albumin has an average half life of only 10 days as compared with about 80 days for other body protein When the organism is exposed to increas d nitrogen breakdown as in high fever widespread necrosis burns and obviously poliomyelitis as well it reacts by a decrease in protein synthesis with a resulting protein deficit This is expressed as a decrease in protein in the whole organism although it is most conspicuous in the serum albumin fraction A cording to calculations made by Elman [3] a decrease in serum albumin

of 1 Gm represents a loss of 30 Gm of protein for the body as a whole Also it must be recalled that such a protein deficit particularly if it is of long duration is invariably accompanied by such vitamin and mineral deficiencies that they must be provided by supplementary nutrition [38]

Although the poliomyelitis virus has long been regarded to have a particular affinity to nervous tissue relatively few data are available on the biochemical changes in the central nervous system in this disease The possibilities of such investigations are obviously limited Moreover the experience gained through analysis of the cerebrospinal fluid is not of such a nature that it does not as yet give any conception of the biochemical changes in the individual nerve cells Nevertheless some observations have b en made which may be of diagnostic value e g with respect to the pyruvic acid content [39 40] phosphatase activity[41] and inorganic P content [4] In addition certain changes in the cerebrospinal fluid have been found in the form of a raised protein content[43] and increased nuclease activity[44] which can be ascribed to some destruction of nervous tissue

Unquestionably there are many other biochemical problems of great interest in poliomyelitis For example it is possible that sialic acid (neuraminic acid) plays an important role for the affinity of poliomyelitis virus to the central nervous system as has been shown for the influenza virus [4 46] Studies of sialic acid-containing mucoproteins inhibiting virus reception in host cells would then be of interest in view of recent discussions regarding the spreading of the poliomyelitis virus in the body

REFERENCES

1 Bower A G Chudnoff J S and Chaney A L Biochemical alterations in acute human poliomyelitis Electrolyte patterns and trends California Med 73 406 1950

2 Bower A G Eaton R M Chudnoff J S Affeldt J E and Chaney A I Patterns of plasma protein changes in acute poliomyelitis patients Treatment with blood plasma Am J M Sc 220 46 1950

3 Chudnoff J S Alterations in serum protein levels in acute human poliomyelitis Rationale for therapy California Med 73 401 19>0

4 Bower A G Morgan F M and Chaney A L Nitrogen and potassium metabolism The reaction pattern in poliomyelitis Am. J M Sc 223 532 1952

5 Chudnoff J S *In* Bower A G (ed) Diagnosis and Treatment of the Acute Phase of Poliomyelitis and Its Complications p 176 Baltimore Williams & Wilkins 1954

6 Astrup P Gøtzche H and Neukirch F Laboratory investigations during treatment of patients with poliomyelitis and respiratory paralysis Brit M J 1 780 1954

7 Astrup P *In* Lassen H C A (ed) Management of Life Threatening Poliomyelitis p 111 Edinburgh Livingstone 1956

8 Astrup P Bennike T and Grandjean L. *In* Lassen H C A (ed) Management of Life Threatening Poliomyelitis p 121 Edinburgh Livingstone 1956

9 Jungner I and Laurent B Biochemical laboratory investigations Acta med scandinav (Supp 316) 154 71 1956

10 Jungner I Lindahl J and Strom J The clinical evaluation of the laboratory investigations Acta med scandinav (Supp 316) 154 80 1956

11 Astrup P and Schrøder S Apparatus for anaerobic determination of the pH of blood at 38 degrees centigrade Scandinav J Clin & Lab Invest 8 30 1956

12 Astrup P A simple electrometric technique for the determination of carbon dioxide tension in blood and plasma total content of carbon dioxide in plasma and bicarbonate content in separated plasma at a fixed carbon dioxide tension (40 mm Hg) Scandinav J Clin & Lab Invest 8 33 1956

13 Jørgensen K and Astrup P Standard bicarbonate its clinical significance and a new method for its determination Scandinav J Clin & Lab Invest 9 122 1957

14 Astrup P Om erkendelsen af forstyrrelser i organismens syreog basestofskifte I II III Ugesk laeger 116 758 19>4

15 Astrup P On the recognition of disturbances in the acid base metabolism Danish M Bull 2 136 1955

16 Astrup P Erkennung der Storungen des Saure/Base Stoffwechsels und ihre klinische Bedeutung Klin Wchnschr 35 749 195/

17 Laragh J H The effect of potassium chloride on hyponatremia J Clin Invest 33 807 1954

18 Earle A M Serum potas ium in poliomye litis J Pediat 36 715 1950
19 Hall R J and Sherman J L Significance of potassium depletion in poliomyelitis Am J Med 14 124 1953
20 Lans H S Stein I F Becker R J Hoyne A L and Meyer K A Potassium deficiency in bulbar poliomyelitis J A M A 146 1017 1951
21 Thomson A E Electrolyte studies in the respiratory paralysis of poliomyelitis Am J Med 22 549 1957
22 Scribner D H Belding H Bogardus G M Fremont Smith K and Burnell J M Potassium intoxication during and immediately following respiratory acidosis J Clin Invest 33 965 1954
23 Jantz H Stoffwechseluntersu hungen bei paroxysmaler Lahmung Nervenarzt 18 360 1947
24 Jungner I To be published
25 Bengtsson I and Nordenstam H Cardiovascular complications in fatal cases of poliomyelitis Acta med scandinav (Supp 316) 154 95 1956
26 Nordenstam H Patho anatomical findings in 26 fatal cases of poliomyelitis Acta med scandinav (Supp 316) 154 114 1956
27 Rindom Schiøtt C The effects of large doses of amino acids on experimental poliomyelitis Danish M Bull 1 185 1954
28 Whedon G D and Shorr E Metabolic studies in paralytic acute anterior poliomye litis J Clin Invest 36 942 1957
a I Alterations in nitrogen and creatine metabolism p 942
b II Alterations in calcium and phosphorus metabolism p 966
c IV Effects of testosterone propionate and estradiol benzoate on calcium phos phorus nitrogen creatine and elec trolyte metabolism p 995
29 Astrup P and Søttrup T In Lassen H C A (ed) Management of Life Threatening Poliomyelitis p 141 Edinburgh Living stone 1956
30 Panel discussion on urolithiasis Urological Survey 4 2 1954
31 Gribetz D Sweet A Y Becker A M Levitt M F and Hodes H L Use of anabolic steroids to reduce hypercalcemia and hypercalciuria caused by immobilization in paralytic poliomyelitis Am J Dis Child 90 612 1955
32 Schulze W Hepatitis als Komplikation der Poliomyelitis Acuta Anterior Klin Wchnschr 30 1075 1952
33 Macciotta A Aspetti clinici e metabolici muscolari della malattia di Heine Medin Ann Ital Pediat 7 374 1954
34 Heilskov N C S and Schønheyder F Creatinuria due to immobilization in bed Acta med scandinav 151 51 1955
35 Hoagland C L Gilder H and Schank R E The synthesis storage and excretion of creatine and glycocyamine in progressive muscular dystrophy and the effects of cer tain hormones on these processes J Exper Med 81 423 1945
36 Milhorat A T and Toscani V Studies in diseases of muscle metabolism of cal cium phosphorus and magnesium in pro gressive mus ular dystrophy myotonia atrophica and familial periodic paralysis Arch Neurol & Psychiat 41 1130 1939
37 Elman R Parenteral Alimentation New York Hoeber 1949
38 Bones C J Nutrition in poliomyelitis J Clin Nutrition 1 355 1952 53
39 Lasch F Über den Brenztraubensaurege halt in Liquor Cerebrospinalis und seine diagnostische Bedeutung Klin Wchnschr 31 941 1953
40 Choremis K Constantinides V and Nicolaides P Pyruvic acid in cerebrospinal fluid during various infectious diseases with central nervous system involvement Ann Pædiat 180 337 1953
41 Colling K G and Rossiter R J Alkaline and acid phosphatase in cerebrospinal fluid Canadian J Res 28 56 1950
42 Odessky L Rosenblatt P Sands I J Schiff I Dubin F M Spielsinger D Kiesenberg J A and Landau L Cerebro spinal fluid inorganic phosphorus in acute poliomyelitis Arch Neurol & Psychiat 73 255 1955
43 Martz C D Spinal fluid protein in polio myelitis and Guillain Barre syndrome Quart Bull Indiana Univ Med Center 15 30 1953
44 Kovacs E Nucleases in cerebrospinal fluid
I Ribonuclease in normal neurologically normal and in pathological CSF s Canad J M Sc 31 437 1953
II Desoxyribonuclease in health and dis ease J Pediat 45 569 1954

III Simultaneous determination of ribo- and desoxyribonuclease in the CSF of patients with poliomyelitis J Pediat 46 691 1955
IV Dephosphorylation of nucleic acids by CSF especially of poliomyelitis patients J Pediat 47 340 1955
45 Gottschalk A Neuraminic acid the functional group of some biologically active mucoproteins Yale J Biol & Med 28 525 1956
46 Gottschalk A Neuraminidase the specific enzyme of influenza virus and Vibrio cholerae Biochim et Biophys Acta 23 645 1957
47 Dunning M F and Plum F Hypercalcemia following poliomyelitis Arch Int Med 99 716 1957

Dr Lewis Dr Spencer's comprehensive report of cardiovascular phenomena associated with severe paralysis is of major medical significance. One important feature is the demonstration again of the complexity of the poliomyelitis lesion in patients who can now be salvaged. His data give a true profile of the incidence and variety of neurologic and myocardial lesions causing circulatory disturbance in severely stricken patients. Fortunately the development of satisfactory methods of care has preserved lives and the parallel growth of diagnostic technics has made it possible to delineate pathologic processes in the ward and clinic rather than in the pathology laboratory.

Dr Spencer's paper leaves little room for comment except commendation and an expression of appreciation for the information which he has abstracted from so large a series of cases and made available. As a passing comment it seems noteworthy that the patients with extreme cardiac and cardiorespiratory abnormalities studied by Dr Spencer's group were frequently admitted to the respiratory center many hours after the onset of life threatening disease. Therefore it is difficult as he pointed out to determine what part of the pathologic process was due solely to the invasion of the poliomyelitis virus and what to the effect of secondary phenomena such as hypoxia. In our own work with patients we have been impressed with the decline in frequency of fulminating disease and extreme disability although by no means its disappearance among those whose care we have been able to supervise from the first stages of illness. There seems to have been progressive increase of salvage potential and diminution of total paralysis among patients who have received co-ordinated medical care from the start. Patients who are transferred to centers only when their lives are endangered by the direct or secondary consequences of poliomyelitis almost invariably already have experienced a significant deterioration of prognosis.

Just as poliomyelitis can produce a wide variety of anatomic lesions and functional disturbances so can it also cause biochemical alterations of great diversity among individuals and seemingly different phenomena among affected populations as well. When the reports of biochemical investigations from Los Angeles Winnipeg Copenhagen and Stockholm are compared there are many similarities but some important differences in the recorded data. In considering these data it is important to bear in mind that the disease under investigation may be different not only because of varying virus virulence and immunity status of the population but also as a result of a multiplicity of other factors. These include the availability of treatment facilities the distances over which patients must be transported and particularly the nature of diagnostic and therapeutic technics which are applied.

With the rapid decline in the incidence of poliomyelitis perhaps many of the problems which have been raised in the past will not be solved. However great contributions already have been made.

Dr Jungner has pointed out forcefully that both respiratory and metabolic influences affect the acid base balance. A question was raised as to whether or not there was a metabolic facet of poliomyelitis which is determined by the character of the virus in different epidemics for example in Copenhagen as opposed to Stockholm. To answer this question it would be necessary to have accurate appraisals of many factors in both epidemics. It would be interesting to have comparative data on carbohydrate administration during the critical phase of illness in such a comparative group of patients. The higher frequency of renal acidosis and elevated nonprotein nitrogen in the earlier Copenhagen epidemic might have been accountable on the basis of marked caloric deficit with increased protein catabolism and possible ketonuria. It may be significant that marked renal acidosis and elevation of nonprotein nitrogen in the blood is not found frequently even among the most severely involved patients under the relatively easy circumstances which prevail in advanced treatment centers in the United States at least during nonepidemic years. Almost certainly such complications would occur if facili

ties and personnel were put to the extreme test of managing outbreaks comparable to those which occurred in the Scandinavian countries in recent years

To maintain the patient with respiratory paralysis at relative homeostasis the principal objectives are well known to be normal arterial oxygen and carbon dioxide tensions and normal pH The methods of achieving this vary from country to country We have had no experience with Dr Astrup s method but similar information is of course obtainable by chemical analysis of the blood by determination of the arterial blood pH by use of the Rahn Fenn diagram for the determination of arterial CO_2 and by various other methods which are all based upon the *Henderson Hasselbalch equation*

With respect to electrolyte balance the complexities of electrolyte phenomena are adequately indicated by Dr Jungner s discussion It is impossible to comment on every detail of the problem which he has so well explored but specific items will be selected for further consideration

The hypokalemia which Dr Jungner attributed principally to increased urinary output of potassium may be influenced by another factor in our opinion Hyperventilation alkalosis has been shown to result in depression of serum potassium The mechanism is probably the shift of potassium ions into cells and the resulting depression of serum potassium level contributes to that which is due to potassium loss in the urine and other fluids such as saliva

Dr Jungner did not stress problems relating to sodium In our experience low sodium syndromes have constituted more difficult metabolic problems than hypokalemia Also this seems to have been the experience in Winnipeg as reported by Thomson He found that low serum sodium values were often encountered in pa
tients with or without evidence o ... reath
ing It was discovered also that ... or
rection by high sodium intake le ... ed
excretion and in some instances t ... s
toration of sodium level in the ser ... y
accomplished with moderate s
The mechanisms involved here are
may i ... cate a true meta
in sor ... litis patients res
i ry ... receptor mecha
nenath

Another and more likely mechanism is hyponatremia due to depletion of intracellular potassium with or without resultant change in extracellular potassium level This intracellular hypokalemia may lead to low serum sodium levels either by intracellular sodium transfer or more likely intracellular to extracellular water shift In either instance total body sodium is normal Under these circumstances administration of additional sodium leads to an increase of extracellular fluid which may prove disastrous The hypotheses stated presuppose a depletion of intracellular potassium i e a loss greater than that which would be accounted for merely by the destruction of muscle The potassium to nitrogen ratio in the urine must be greater than *the potassium to nitrogen ratio of muscle tissue*

Dr Jungner referred briefly to the relationship between calcium and phosphorus metabolism and the formation of calculi in the urinary tract which has been investigated by Dr James Elliot and our other associates in our laboratory The magnitude of the clinical problem is well known in the extent of the metabolic derangement as indicated by the observations of Plum and Dunning and demonstrated prolonged calcium loss even in relatively mild bulbar paralysis

By means of petrographic analysis with the polarized light microscope it has been possible to identify the mineral content of calculi occurring in recumbent patients Struvite and apatite are the commonest constituents occurring in poliomyelitis Almost all of the renal and bladder stones are composed of these two minerals that is basic or tertiary calcium phosphate and magnesium ammonium phosphate Approximately 5 per cent are calcium oxalate An interesting fact concerning the formation of stones is the relationship between solubility of calcium phos hate and pH of the urine In
simple aqu ... on calcium phosphate pre
cipitates as ... pH 6 2 or higher Apatite
precipitates ... e at pH 6 6 or higher
Brushite ... tant compound is found
at lower ... t rarely occurs in kidney
stones w ... you begi ... find apatite
precipitati ... the hi ... levels
struvite ... is of ... rtance
cause it ... ry c ... ship to
inary p ... ve ... solid or
tallin ... impor

tance Above pH 7.2 struvite crystallizes from urine which is saturated or supersaturated with calcium and phosphate These findings indicate that in addition to hypercalciuria an abnormally high urinary pH must be maintained for apatite or struvite calculi to develop The mean pH of normal 24 hours urine is 6 1

Our work has demonstrated that the commonly held view that urine is a supersaturated solution of calcium phosphate is not true In most normal persons the urine is undersaturated with calcium phosphate and this is true also of many patients with severe paralysis due to poliomyelitis However normal urine may be supersaturated as evidenced by the finding of brushite and apatite crystals in normal urines Likewise the urine of paralyzed patients may be and often is saturated or supersaturated with calcium phosphate Factors which favor the precipitation of calcium salts for the formation of calculi are the high pH mentioned above the presence of foreign bodies such as catheters and the presence of infection In some instances infection with urea splitting organisms is the basis for the elevated pH but this is by no means always the case As a matter of fact the mechanism for persistent alkaline pH s is not well understood

Protein metabolism is a subject which warrants a great deal of discussion and further investigation Our own studies have been concerned principally with determination of creatinine clearances and creatinine phosphate ratios In late convalescent poliomyelitis we found a consistent reduction of serum creatinine with levels of 0 2 0 6 mg 100 ml as compared to normal values of 1 0 1 5 mg/100 ml in controls Endogenous creatinine clearance also has been found to be impaired Whether this is due to a renal lesion or a defect in creatinine metabolism remains to be determined

The empirical observation of a significant fall in serum albumin level during the initial few weeks of illness is interesting We have not investigated this problem However the interpretation of the fall of serum albumin level is extremely difficult Without an estimation of albumin pool size and a knowledge of average half life no deductions concerning albumin turnover are possible In one patient in late convalescence studied in our laboratory by use of labeled serum albumin there was no demonstrable abnormality of albumin turnover Attention should be called to the fact that various methods of labeling albumin lead to different estimations of half life and pool size The 10 day half life mentioned by Dr Jungner is now considered by almost all workers in the field to be short by 5 to 15 days

BIBLIOGRAPHY

Burnell J M Zillamil M Uyeno B T and Scribner B H Alterations in the internal equilibrium of potassium induced by acid base disorders in humans (abstr) Clin Res Proc 4 715 1956

Danomski D S Fergus E B and Mateer F M The low salt syndromes Ann Int Med 43 643 1955

Dunning M F and Plum F Hypercalciuria following poliomyelitis Arch Int Med 99 716 1957

Gabardi Aldo and Davenport H W An improved device for obtaining plasma anaerobically J Lab & Clin Med 34 1169 1949

Margen S The deiodination of iodinated serum albumin Ann New York Acad Sc (In Press) (This monograph of the New York Academy of Science will contain recent data on determination of half life of human serum albumin)

Radford E P Jr Ferris B G Jr and Kriete B C Clinical use of a nomogram to estimate proper ventilation during artificial respiration New England J Med 251 877 1954

Rahn H and Fenn W O A graphical analysis of respiratory gas exchange the O_2-CO diagram *in* Comroe *et al* The Lung p 190 Chicago Yearbook Pub 1955 Monograph and charts publ by Amer Physiol Soc Washington D C 1955

Thomson A E Electrolyte studies in the respiratory paralysis of poliomyelitis Am J Med 22 549 1957

Dr Sanz I shall discuss one topic of the remarkable report of Dr Jungner namely the control of artificial respiration

Using a blood pH electrode that permits the determination of the blood pH with very small quantities of blood we have studied the relationship of capillary and arterial blood and the interdependence of blood pH and total CO_2 of serum in purely respiratory cases metabolic cases and cases with respiratory and metabolic disturbances The blood pH measurement is extremely delicate because of the little changes that

might be significant The determination must be correct to 1/100 of a pH unit and contact with air must be strictly avoided during the whole procedure Also all anticoagulants including oxalate and heparin must be avoided because of their acidifying effect upon the blood pH which is enhanced in hypoproteinemia In addition the temperature of the blood should not undergo any change at any time We regulate the temperature of the electrode to exactly the patient s temperature within 1/10° C and measure the blood pH within a few seconds as a routine bedside determination Blood (5 to 10 microliters) is drawn into the thermostabilized and carefully shielded capillary electrode which is regenerated after each measurement by drawing pepsin solution into it The regeneration is accomplished in 2 minutes Before and after the measurement the electrode is checked with at least 2 certified buffer solutions of which the values are known to 1 100 of a pH unit at different temperatures

The total CO content is measured by the microgasometer of Natelson permitting a determination in 30 microliters of serum

In 20 patients capillary blood closely approached arterial blood with respect to pH and CO when a good hyperemia of the ear lobe or fingertip is ascertained The first drop is always discarded

All normal subjects had pH values of 7 38 to 7 42 whereas the venous blood shows pH values lying 0 02 to 0 03 pH units lower than the corresponding arterial or capillary blood values

In purely respiratory acidosis CO accumulates and the pH values fall very rapidly The critical values according to our experiences are pH 7.20 to 7.22 At this moment artificial respiration must be installed if the pH falls below this limit irreparable damage of the respiratory centers occurs On the other hand metabolic acidosis may show much lower blood pH and values of 7 07 to 7 10 have been observed in uremia which seemed well tolerated In these cases the total CO is far below normal Therefore it is extremely important to differentiate between respiratory and metabolic disturbances both of which may occur in poliomyelitis For this reason the total CO must be determined and the CO tension calculated according to Henderson and Hasselbalch

Practice has shown that in purely respiratory cases mainly in the acute phase of poliomyelitis blood pH values are a sufficient guide for the equilibration of the patient especially as they can be repeated easily very often The logarithm of the CO tension shows a linear relationship to the blood pH

It is of extremely high importance to reach respiratory equilibrium as soon as possible i e in the very first hours of respiratory disturbance and to maintain very carefully this equilibrium The virus infection we believe reaches higher centers in inadequate respiratory conditions than when the patient is perfectly equilibrated and of course oxygen given in a sufficient amount A slight hyperventilation is preferable to an even small hypoventilation where traces of CO are accumulated with every breath

Pulmonary Complications in Poliomyelitis

DR HANS NORDENSTAM

Patho-anatomi ally the pulmonary complications in poliomyelitis present only a few types In a natural way they can be divided into 3 groups

1 Complications following respiratory paralys and/or airway obstruction The conditions constituting this group seem to be causally connected and are aspiration emphysema atelectasis and petechiae from suffocation

Many authors state that widespread atelectasis develops frequently in respiratory poliomyelitis in spite of adequate respirator treatment and that it is the main cause of death in a number of cases The results of the controlled respiration not quite as successful as expected have been explained inter alia by paralysis of the ciliary movements in the mucous membranes of the bronchi out of reach of the suction tube and of the usual bronchial cleansing In addition the secretion is assumed to be displaced still more toward the periphery through the variations of the respirator pressure and finally fill out the alveoli thus interfering with the exchange of the gases In the present material genuine atelectasis could be diagnosed pathoanatomically in only 8 cases

Table 1/4 shows all permanent complications and the cases are ranked according to clinical symptoms

2 The second group consists of mechanical injuries and inflammatory changes

I must talk about one type of inflammatory change namely interstitial pneumonitis In the monograph of 1956 we had low microscopic incidence of bronchopneumonia Microscopically we found about three times as much as macroscopically This is one of the reasons for a new investigation which was made this year and

TABLE 1/4 INCIDENCE RATES OF PULMONARY COMPLICATIONS RELATED TO OTHER ORGAN SYSTEMS AND TO DIFFERENT CLINICAL GROUPS

	RESPIRATORY SYSTEM												REMAINING ORGAN SYSTEMS			
Clinical groups	Atelectasis	Emphysema	Aspiration	Suffocation hemorrhages	Decubital ulcers in hypopharynx and trachea	Tracheobronchitis	Bronchopneumonia of conventional type	Interstitial pneumonitis	Extravasation of erythrocytes into the alveoli	Macrophages containing hemosiderin like pigment	Pulmonary edema	Hydrectasis	General and r ventricle dilat Hypertrophy of r heart ventr	Myocarditis	Pulmonary embolism	Erosions of gastric mucosa acid malacia in esophagus/stomach
I Ia	1 2	2 1	0 2	5 1	3 2	4 3	1 1	10 2	10 3	10 2	10 2	10 2	10 3	10 3	1 0	3 3
II	3	6	1	5	4	8	5	7	7	6	8	4	8	8	2	5
III	2	5	3	4	0	4	0	4	5	4	3	0	2	5	0	3
Totals	8	14	6	15	9	19	7	23	25	22	23	16	23	26	3	14

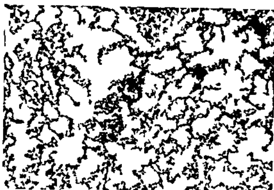


Fig 330 Hydrectasis and capillary hyperemia (van Gieson s stain) (×20)

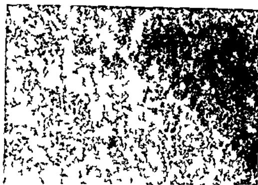


Fig 332 Interstitial pneumonitis and hydrectasis Large amounts of erythrocytes in the alveoli (van Gieson s stain) (×20)

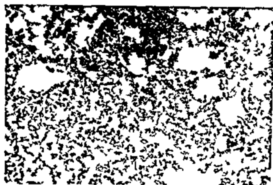


Fig 331 Interstitial inflammatory infiltration of the lung Marked capillary hyperemia and a few erythrocytes in the alveoli (hematoxylin-eosin stain) (×20)

now inflammatory changes could be demonstrated in ?3 cases out of 26 fatal cases of poliomyelitis Also we could differentiate between 2 types of inflammatory alteration namely patchy bronchopneumonia of conventional type and interstitial changes simulating virus pneumonitis Microscopically we had 7 bronchopneumonia cases which showed the ordinary varying appearance the alveoli engorged with fibrinous exudate and inflammatory cells above all polynuclears

The interstitial or pneumonitis type was observed in 23 cases Only microscopic study will disclose it and mild degrees will be concealed easily by more conspicuous phenomena namely excessive erythrocytic engorgement of the alveoli extreme dilatation of the capillaries in the alveolar walls emphysema or atelectasis The interstitial infiltrations are often closely interwoven with intra alveolar fibrinous exudate and abundant polynuclear leukocytes

Histologically the interstitial infiltrations resembling those of several types of virus pneumonitis are mainly composed of lymphocytes and monocytes with very few polynuclears The infiltrations show an arrangement in clusters and streaks around vessels and nerves partly following fine bronchial branches which appear encased in the same way by lymphocytes and monocytes However the infiltration ceases in the peripheral parts of the bronchial wall and the remaining layers present no signs of inflammation unless a coexisting purulent bronchitis is demonstrable The vessels of the alveolar walls show excessive dilatation often giving an erroneous impression of general thickening of the walls due to other causes Large numbers of erythrocytes intra alveolar as well as interstitial and abundant macrophages containing pigment similar to hemosiderin occur frequently presumably owing to the extreme dilatation of the vessels and extravasation of red cells Figures 330 and 331 show interstitial pneumonitis with heavy pulmonary edema dilatation of capillaries in the intra alveolar walls Figure 33? also is an interstitial pneumonia or pneumonitis with a great many red blood cells intermingled in the alveoli

3 The third group are called hydrodynamic disorders in the peripheral respiratory tract Patho-anatomically this group is an entity consisting of pulmonary edema only as mentioned However it is an important group quantitatively and yields interesting information concerning the character and pathogenesis of poliomyelitis Not until recent years has the hydrodynamic disorder in itself been considered an important

complication Simultaneously the attitude toward them is undergoing a change at present they are looked upon by many as a result of the poliomyelitis directly equivalent to the paralysis of striated muscles rather than a secondary complication However this interpretation of the pulmonary edema is to a great extent a revival of the former concept of damage to cerebral autonomic centers

Before the era of antibiotics and controlled respiration pneumonia and atelectasis were the most important pulmonary complications as to incidence (as well as cause of death) After the introduction of modern antibiotic treatment atelectasis was said to constitute an overwhelming group and as regards fatalities to rank foremost beyond comparison Great expectations were aroused by the improvement of the respirator technic and of the management of controlled respiration However pulmonary edema was reported as early as in the 1930 s but practically only in connection with pneumonia atelectasis encephalitis and after 1941 myocarditis There are quite remarkable differences as to incidence rates in series of otherwise similar cases of fatal poliomyelitis A brief account will be given of three theories on the pathogenesis of pulmonary edema in poliomyelitis These are (1) paralysis of cerebral autonomic centers (2) atelectasis (3) the cardiovascular pathogenesis

In a previous paper I have called the pulmonary edema in poliomyelitis hydrectasis because of its differences from ordinary pulmonary edema It is rather difficult to explain this high degree of edema but the demonstration of chromaffin granules in characteristically shaped cells in human skins and other tissues makes possible a different explanation not discussed previously Next to the skin pulmonary tissue has hitherto showed the greatest proportion of these cells Also in vessels arteries and veins as well as capillaries they occur rather abundantly In human skin the cells show long protrusions containing the chromaffin granules We have found that these granules can go away when the skin is irritated in some way and we have made injections of different things such as histamine and the histamine releaser P 48 80 Then we can see how the granules go away for some minutes

The experiences described provide the basis for a different pathogenetic concept of pulmonary edema and hydrectasis in poliomyelitis It may arise from capillary leakage caused by essentially peripheral neuromuscular paralysis due to the interstitial pneumonitis throwing the transmittors out of function with subsequent blockage of constricting impulses to the vessels The chromaffin cells having neural character embryologically could reasonably be invaded by the poliomyelitis virus in accordance with its neurotropism or predilection for structures deriving from the neuroderm

BIBLIOGRAPHY

Adams Ray J and Nordenstam H Lyon Chir (Vol memorial du Professeur Rene Leriche) 1956

Bengtsson E and Johnsson T Cardiologia 20 65 1952

Bengtsson E and Nordenstam H Acta med scandinav (Supp 316) 154 95 1956

Bengtsson E and Werneman H Acta med scandinav (Supp 316) 154 55 1956

Berglund E Am J Physiol 178 381 1954

Berglund E Sarnoff S J and Isaacs J P Circulation Res 3 133 1955

Debre R (editor) Poliomyelitis WHO Publication No 26 1955

de Jong B J and de Haan J Acta Neerl Morphol 5 26 1943 45

Denhardt A Ztschr Kreislauff 42 159 1953

Fanconi G Triangle 2 249 1956

Gould S E (editor) Pathology of the Heart Springfield Ill Thomas 1956

Hildes J A Schaberg A and Adcock A J E Circulation 12 986 1955

Larson C P Northwest Med 40 448 1941

Liszkai L Acta Morphol 5 385 1955

Nordenstam H and Adams Ray J Ztschr Zellforsch 45 435 1957

Phodin J Adams Ray J and Nordenstam H To be published

Sarnoff S J and Berglund E Am J Physiol 170 588 1952

Sarnoff S J Berglund E and Sarnoff C L J Applied Physiol 5 367 1953

Sarnoff S J and Sarnoff L C Dis of Chest 22 685 1952

Strom J Acta med scandinav (Supp 316) 154 40 1956

Teloh H A Arch Path 55 408 1953

Trueta J Wilson K and Agerholm M Handbook on Poliomyelitis Oxford Blackwell 19 6

Uflacker H Arch Kinderheilk 149 144 1954

Gastro-Intestinal Complications in Poliomyelitis

DR HAROLD N NEU

Although the *reports in the literature of the* effect of the poliomyelitis virus on the neuro muscular system are voluminous few concern gastro intestinal complications In fact in the *Bibliography of Infantile Paraly is* from 1789 1944 with over 8 000 references collected there are no reports of complicating gastro intestinal ulcerations The recent reports have largely *been concerned with the complications seen in* the acute bulbar poliomyelitis

Saphir has emphasized that there is no gastro-intestinal lesion characteristic of poliomyelitis The gastro intestinal lesion which occurs in acute bulbar poliomyelitis probably results from damage to the hypothalamus The association between brain damage and changes in the stom ach was of course first noted by Kammerer in 1821 and later by Rokitansky in 1841 The experimental investigators have as early as 1867 and since confirmed that damage to the anterior portion of the hypothalamus involving the tuberal region results in gastric hemorrhage and erosion Furthermore its destruction results in the impairment of parasympathetic activity with *altered digestion inhibited peristalsis* and marked distention

Dr Baker of the University of Minnesota amplified the existing knowledge by detailed study of 115 autopsied cases of bulbar polio-myelitis from which the complete hypothalamus was available 16 of these patients had mani fested gastric bleeding during their acute illness *and at the same time Dr Baker and his group* noted that 61 per cent of the patients with bulbar poliomyelitis had some destruction of the hypothalamic nuclei

All observers agree that in the patients with acute poliomyelitis presence of these gastro-intestinal complications must be regarded as a grave omen seriously affecting the prognosis for the patient

In this study we have been more interested in the gastro intestinal complications of polio-myelitis patients respiratory and postrespirator in the convalescent and chronic phases In order to evaluate the extent of these complications the applications for admission to the respirator and rehabilitation centers of the United States during

TABLE 175 GASTRO INTESTINAL FUNCTION OF ALL APPLICANTS FOR ADMISSION TO REGIONAL *RESPIRATORY AND REHABILITATION CENTERS FEBRUARY 1955 TO FEBRUARY 1957**

COMPLICATIONS	TOTAL ALL APPLICANTS	PATIENT CAN SWALLOW			
		NOTHING	LIQUIDS	SEMI SOLIDS	SOLIDS
Total all applicants	529	29	19	59	422
None	441	2	7	47	385
Gastric tube	39	22	8	6	3
G I bleeding	6		1		5
Gastric dilatation	22			3	19
Gastric tube and G I bleeding	3	3			
Gastric tube and gastric dilatation	5	1	2		2
G I bleeding and gastric dilatation	12	1		3	8
All three	1		1		

Based on appl cations for adm ss on t Region l Re piratory C nt r (NFIP F rm 64) recei ed d g the p riod Februa y 1955 to Feb ary 1957

TABLE 176 GASTRO-INTESTINAL FUNCTION OF ALL APPLICANTS FOR ADMISSION TO REGIONAL RESPIRATORY AND REHABILITATION CENTERS FEBRUARY 1955 TO FEBRUARY 1957 DIVIDED INTO THOSE UNDER 15 YEARS AND THOSE OVER 15 YEARS*

COMPLICATIONS	TOTAL, ALL APPLICANTS	PATIENT CAN SWALLOW			
		NOTHING	LIQUIDS	SEMI-SOLIDS	SOLIDS
Total Applicants Under Age 15	91	8	2	12	69
None	76	1		10	65
Gastric tube	9	5	2	2	
G I bleeding					
Gastric dilatation	3				3
Gastric tube and G I bleeding	1	1			
Gastric tube and gastric dilatation	1	1			
G I bleeding and gastric dilatation	1				1
All three					
Total Applicants Aged 15 and Over	438	21	17	47	353
None	365	1	7	37	320
Gastric tube	30	17	6	4	3
G I bleeding	6		1		5
Gastric dilatation	19			3	16
Gastric tube and G I bleeding	2	2			
Gastric tube and gastric dilatation	4		2		2
G I bleeding and gastric dilatation	11	1		3	7
All three	1		1		

B sed on appl ations f r adm ssion to Regional R p at ry Ce t r (NFIP Fo m 64) ecei d dur ng the per od F b ry 1955 to F b uary 195

the 25 month period of February 1955 to February 1957 were re iewed (Table 175) There were over 529 applications which included a small number (less than 5) of multiple applications which were received from patients who had applied to more than one center The analysis is of course complicated but those applications which did not specify the gastro-intestinal status of the patient were excluded from the tabulation In summary 50 per cent of the patients had some type of gastro intestinal complication from the onset of their illness Only 9 per cent of them had the gastric tube at the time of application Further in Table 176 when these are broken down between the ages of 15 and under and those who are 15 and over approximately the same proportions (84 % and 83) reported neither gastric tube gastro intestinal bleeding nor gastric dilatation Since most of these patients were already in the chronic and convalescent phase before application to these centers these statistics clearly indicate that in the chronic poliomyelitis patient gastro-intestinal complications are not as frequent as they are seen in acute poliomyelitis

In our own Respiratory and Rehabilitation Center we studied 141 respirator or postrespirator patients (Table 177) These patients were chiefly bulbospinal or high spinal types of poliomyelitis All were in the convalescent or chronic

TABLE 177 GASTRO-INTESTINAL COMPLICATIONS IN 141 RESPIRATOR AND POSTRESPIRATOR PATIENTS ADMITTED TO A RESPIRATORY CENTER

Total No Admissions	141	100%
Peptic ulcer	2	1 4%
Hemorrhage	5	3 5%
Acute gastric dilatation	8	5 6%
Distention and stasis	24	17 0%

Tw f th se wer the l r case



FIG 333 Severe dilatation

phase with over 80 per cent in the chronic phase The onset of their acute illnesses had been from 1 month to 6 years prior to admission to the Center In age they varied from 6 years to 49 years although the majority were 15 30 years of age

In the respirator group of patients the vital capacities ranged from 0 to 900 cc In the postrespirator group they were over 1 000 cc but usually under one third of normal predicted vital capacity

Interestingly only 3 patients had an indwelling gastric tube on admission to the Respiratory Center The remaining patients could swallow liquids semi solids or solids although actually the residual paralysis of the palate and pharyngeal muscles in many patients made mealtime a slow and laborious effort both for the patient and the attendant Of course since the respiratory problems were paramount an appropriate program was instituted to wean the patients to lesser respiratory aids as soon as feasible and of course it is somewhat in retrospect that we can appraise the gastro intestinal complications which arose

It may be noted in this table that the 2 cases of peptic ulcer represent an incidence less than that which occurs in the normal population of this age group All of these patients were required to make adjustments to situations of catastrophic stress yet in only these 2 were we able to confirm the existence of ulcer The management in these two cases was uneventful

Massive gastric hemorrhage with hematemesis was not encountered However in 5 cases there was sufficient bleeding to produce melena which was confirmed by guaiac tests 2 of the patients were mentioned previously Routine stool studies for occult blood were not performed However since all of these patients were bedfast and over 80 of them were quadriplegics in addition to the respiratory involvement daily reporting of the gross characteristics of the stools was routine

In our series of patients 8 had acute gastric dilatations of an emergency nature requiring immediate use of a gastric tube with suctioning In all patients the onset was unnoticed until the symptoms became acute with definite impairment of respiratory function In 3 of the cases there had been food indiscretion such as the following

A 26-year-old white housewife who had been maintaining her respiratory status at home with a cuirass respirator and rocking bed despite her vital capacity of 500 cc one evening ate some hamburger with her family The other members developed nausea and vomited The patient however was unable to vomit She experienced abdominal distention which was so severe it interfered with the successful operation of the cuirass respirator She showed progressive respiratory embarrassment and was admitted to the Center On admission severe gastric dilatation was observed A Levine tube was passed left in place for 24 hours intravenous fluids administered 36 hours later she returned to her home Figure 333 illustrates the severe dilatation and this has been observed many times in other patients

Fortunately the awareness of this complication in the respirator patient results in early treatment when the beneficial effects are most apparent In none of our cases did the problems of severe electrolyte disturbance arise The elevation of the diaphragm by the dilated stomach impedes respiration to such a degree that acute

respiratory failure may supervene Dr Baker has felt that many of the delayed deaths of bulbar poliomyelitis may be due to this acute dilatation Certainly we have suspected that in a number of unexplained sudden deaths of chronic respirator patients in home care situations this may have been a factor

The most common gastro-intestinal problems in the chronic respirator patient are related to simple stasis and distention This is evidenced by the frequency with which constipation is noted in a Respiratory Center by the difficulty in securing satisfactory scout films of the abdomen for survey of renal calculi Dehydration the low phosphorus acid ash diet may contribute to the problem but more important is the absence of effective abdominal muscle contraction with increase in the intra abdominal pressure

The immobility did not appear to be significant since impactions were noted more frequently in those patients who were up in wheel chairs as compared with the quadriplegics confined to the full body respirator or to the rocking bed In 70 per cent of our patients this was sufficiently important a problem to merit active attention of the staff

It is difficult to determine whether these complications represent residual effects of damage to the hypothalamus or were side-effects of the difficult psychological adjustments required in facing a catastrophic illness Drugs such as Prostigmin or Pitressin did not have their usual effects in increasing peristalsis in these patients

When fecal impactions do occur it is important not to exhaust the patient in removing the impacted stool The use of oil retention enemas has not been any more successful than the use of fecal softener (such as dehydroxy anthroquinone or dioctyl sodium sulfosuccinate) in preventing impaction In none of our patients had mechanical obstruction requiring surgery occurred

In conclusion we feel that our experience and study emphasizes that the gastro intestinal complications usually do not have the bad prognosis in chronic respirator patients that such complications imply in the acute phase of poliomyelitis One exception is acute gastric dilatation which is definitely more frequent in the respirator patient than in other chronic disabling illnesses It is impossible on the basis of our experience to postulate that this complication is a residual of injury to the hypothalamus with resultant altered parasympathetic balance yet the residuals of bulbar involvement exist in many of these patients This observation is contrary to the frequently accepted concept that recovery from bulbar poliomyelitis is usually complete

Careful day by-day observations are equally important to the chronic respirator patient in order that gastro-intestinal complications do not further impair the already diminished respiratory reserve

BIBLIOGRAPHY

Baker A B Brown J A and Cornwell S Poliomyelitis VII Gastro intestinal disturbances their relationship to lesions in the hypothalamus J Nerv & Ment Dis 116 715 1952

Baker A B Cornwell S and Brown I Poliomyelitis VI The hypothalamus A M A Arch Neurol & Psychiat 68 16 1952

Bibliography of Infantile Paralysis 1789 1944 Philadelphia Lippincott 1946

Bockus H L Gastroenterology vol 1 chap 40 Philadelphia Saunders 1944

Burdenko N and Mogelnitzki B Zur Pathogenese einiger des runden Magen Darmgeschwurs Ztschr des Neurol u Psychiat 103 42 1926

Cook C D Hartman J R Sarnoff S J and Berenberg W Gastro intestinal lesions in acute bulbar poliomyelitis report of five cases with recovery in one Pediatrics 7 415 1951

Cushing H Peptic ulcers and the inner brain Surg Gynec & Obst 55 1 1932

Dragstedt L R Acute dilatation of the stomach *in* Practice of Surgery vol 6 Baltimore Prior

Erskine F A Mason J H Jr and McDade H Perforation of esophageal ulcers in bulbar poliomyelitis—report of 2 fatal cases Am J Med 8 239 1950

Heyde E C and Robinson S Acute peptic ulceration accompanying bulbar poliomyelitis—report of 2 cases Gastroenterology 11 519 1948

Hoxsey R J Gastro intestinal hemorrhage as a complication of poliomyelitis A M A Arch Int Med 92 662 1953

Plum F Personal communication

Rokitansky C A Manual of Pathologic Anat

omy Trans by W E Swaine Philadelphia Blanchard and Lea 1885
Saphir O Visceral lesions in poliomyelitis Am J Path 21 99 1945
Schiff M Lecons sur la physiologie de la digestion Florence and Turin Loescher 1867
Sheehan D The hypothalamus and gastro intestinal regulation a review Nerv & Ment Dis Proc 20 589 1940
Stickle G Personal communication
Watts J W and Fulton J F Effect of lesions of hypothalamus upon the gastro-intestinal tract and heart in monkeys Ann Surg 101 363 1935

DISCUSSION

DR NEUKIRCH In addition to the question of gastro-intestinal complications our experience permits some further information on the incidence of peptic ulcers although they are encountered only rarely in the acute stage of the disease Among the 115 patients with poliomyelitis who died in 1952 in the Blegdamshospital Copenhagen autopsy revealed peptic ulcers in 8 cases (6 men and 2 women) in these 8 cases death occurred within periods ranging from 3 to 27 days after the onset of the disease Melena and hematemesis had only been recognized in 1 of the cases whereas the remaining 7 peptic ulcers represented casual autopsy findings the complication had *not* manifested itself in gastro intestinal hemorrhage Stool samples were not examined chemically for blood by routine during the acute stage of the disease Severe lesions of the brain stem particularly of the hypothalamus were demonstrated in all 8 cases

A particular interest is attached to 5 patients (out of a total of 349) with respiratory insufficiency in whom massive gastro intestinal hemorrhage was present i e 1 case of hematemesis and 4 of melena the peptic ulcer just dealt with was found at autopsy of 1 of these patients autopsy findings from another of the patients showed no intestinal abnormalities The 3 survivors 2 of whom were infants did not present any signs of gastro intestinal pathologic conditions In this connection attention should be called to findings (Bennike and Grandjean) demonstrating that manifest hemorrhagic diathesis may represent a not infrequent complication in patients severely stricken by poliomyelitis particularly if combined with respiratory paralysis (15 / out of a total of 349 cases) Most likely this increased bleeding tendency may be ascribed to a reduced capillary resistance because this was the only demonstrable abnormality found by investigations on the hemorrhagic diathesis Such reduced capillary resistance the origin of which remains obscure was also demonstrated in some patients in whom there were no signs of hemorrhagic diathesis

This hemorrhagic tendency in patients with poliomyelitis may manifest itself by bleeding from the skin or from the various mucous membranes Hence it is justifiable to interpret intestinal hemorrhages merely as one manifestation of a general hemorrhagic diathesis and not necessarily due to a peptic ulcer Besides no connection between hypertension and hemorrhagic tendencies has been observed

Although patients suffering from acute poliomyelitis with respiratory paralysis are exposed to severe stress the incidence of peptic ulcers must be considered low and there is no evidence that these patients are more apt to get a peptic ulcer than other people irrespective of the degree of severity of their poliomyelitic sequel This does not seem to be suggestive of a decisive importance of stress—at least not of brief stress—as regards the pathogenesis of peptic ulcers

During the acute stage of the disease paralytic ileus and gastric dilatation represent the most commonly encountered gastro intestinal complications as regards the latter the dilatation caused by artificial ventilation cannot be excluded as a contributory factor

During the chronic stage of the disease constipation and fecal impaction represent the most frequent complications The fecal impaction is occasionally severe but evidently causes no pains —probably because of the insufficient intestinal motility However the mechanical ileus represents an extremely rare complication at this stage of the disease

BIBLIOGRAPHY

Bennike T and Grandjean L C Hemorrhagic diathesis in poliomyelitis Acta med scandinav 155 225 1956

Genito-Urinary Complications in Patients Severely Stricken by Poliomyelitis

DR AVRON Y SWEET

The sole urinary tract abnormality in acute poliomyelitis is the retention of urine which results from temporary paralysis of the urinary bladder This small irksome problem plays an important role in the pathogenesis of urinary tract infections calculus formation and in some cases ultimate renal failure during the post acute period in severely involved patients

Most patients with severe poliomyelitis particularly those with involvement of the lower extremities have paralysis of the urinary bladder and many of these obtain relief with Furmethide physostigmine or Urecholine Those who are not helped must be catheterized This act together with the continued presence of the catheter in the bladder is invariably responsible for infection in the bladder and in time ascending infection of the urinary tract

Hypercalciuria is another important factor in the production of serious urologic complications in the postacute period in severely involved patients It is commonly held that the flaccid paralysis and lack of weight bearing result in a diminution of protein matrix upon which calcium is normally deposited Therefore the calcium which ordinarily would be deposited on that protein matrix remains in the blood and is ultimately excreted by the kidneys The resulting hypercalciuria is usually in the magnitude of 3 to 4 times the normal daily excretion of calcium Dunning and Plum have demonstrated hypercalciuria in patients with bulbar involvement only and suggest that it results from some central neuronal abnormality

Bacterial infections of the urine play a villainous role in the production of complications of the urinary tract for regardless of the offending organism pus tends to increase the pH of the urine Hypercalciuria and alkaline urine favor the precipitation of calcium in the urinary bladder rather early in the disease Usually incrustations form about the indwelling catheter Later the same circumstances except for the presence of the catheter exist in the pelvis of the kidneys

The incidence of urinary tract calculi apparently varies according to the degree and extent of paralysis but data are not available which clearly show this nor do we know the influence of respiratory abnormalities duration of bed rest and prophylactic measures An incidence of 4 8 per cent of all paralytic cases in adults was reported by Rodgers and his co-workers however stones occur in 18 per cent of all patients who require respirators irrespective of the duration of respiratory assistance The incidence of stones is much lower in children about 1 per cent At the Poliomyelitis Respirator Center at the Mount Sinai Hospital in New York where patients have been admitted from 1 to 6 months following the onset of illness we have found the incidence of calculi of the urinary tract to be 40 per ent We have not found any patient with stones to be free of infection Neither has any patient with mildly involved or normal lower extremities irrespective of respiratory inadequacy extent of paralysis elsewhere or duration of confinement to bed been found to have calculi

With improved materials and technics in care and rehabilitation an increase has occurred in the salvage rate and longevity of severely involved poliomyelitis victims As a consequence the long term complications and intercurrent problems have attained great importance The most frequently occurring difficulty is that of intercurrent respiratory infections The next most common are the urinary tract diseases which include exacerbations of pyelitis and pyelonephritis partial and complete obstructive uropathies development of staghorn calculi and resultant diminution of renal function Although respiratory infections are most frequent urologic complications are responsible for more

time lost from the standpoint of prolongation of hospital stay removal from active rehabilitation programs and readmissions to hospitals In our experience urologic surgical procedures have outnumbered all other operations combined and are in general of a more serious nature By 1948 the medical literature recorded but five respirator patients who had survived major surgery Today major urologic surgery is not uncommonly performed upon severely involved respirator patients in whom postoperative complications are unusual and deaths are a rarity In the main these procedures are ureterolithotomies pyelolithotomies and combined pyeloureterolithotomies Excellent results have been obtained because of experienced surgeons careful choice and administration of anesthetic agents and meticulous postop rative are Because of the lability of the blood pressure in respirator patients they must be positioned on the operating table slowly and gently while blood pressure determinations are made frequently Anesthetic agents which are irritating to the respiratory tract are to be avoided Ventilation is maintained through an endotracheal tube by manually squeezing an anesthesia bag or by an intermittent positive pressure machine Usually surgery is complicated only by considerable oozing of blood which has been attributed to failure of the capillaries to contract because of perivascular fibrosis Postoperatively careful control of respiration attention to respiratory tract secretions and judicious fluid and electrolyte therapy are imperative

The diagnosis of obstructi e uropathy may be difficult for frequently severely involved patients do not complain of the prime symptoms of ureteral or ureteropelvic obstruction namely pain This has been attributed to involvement of the lateral columns of the spinal cord The patient may complain only of abdominal discomfort caused by ileus This occurs so frequently that we always obtain an excretory urogram when a patient complains of abdominal discomfort Although patients with urinary tract calculi in our experience invariably have infection they often do not have fever associated with obstructive uropathy Leukocytosis may or may not be present

To prevent or minimize the deleterious effects of hypercalciuria and urinary tract infections attempts should be made to control these factors during the acute phase of poliomyelitis If catheterization of the bladder is done prophylacti antibiotic therapy should be given in therapeutic doses Chloramphenicol is probably the most desirable drug inasmuch as it is effective against many of the gram negative organisms as well as staphylococci Irrigation of the bladder twice daily with Suby s solution may prevent the formation of bladder calculi Removal of the bladder catheter at the earliest time possible is important Fluid intake should be sufficient to produce a satisfactory daily urine volume We have found that the administration of anabolic steroids principally androgens results in a 40 per cent diminution in the daily urinary loss of calcium in severely involved poliomyelitis patients Therefore it would seem that androgen therapy might very well be added to the therapeutic regimen during the a ute stage of poliomyelitis

Following the acute phase of the illness androgen therapy should be continued a satisfactory urinary volume should be maintained and if the bladder catheter has not been removed previously vigorous attempts should be made to do so Antibiotic therapy should be continued until after the catheter has been removed and cultures of the urine are sterile It has been suggested that rocking beds and tilt tables are beneficial in diminishing hypercalciuria however balance studies have shown that these modalities are useless Rocking beds and tilt tables have been recommended also for the postural drainage of the upper urinary tracts There is no evidence that this is necessary or that the devices used prevent stasis Many authors urge the use of acid ash and low calcium diets while others suggest the administration of aluminum hydroxide gel in order to decrease phosphorus and calcium absorption from the bowel There is no evidence to support the usefulness of these measures Recently the use of salicylates for the prevention of calculi has been advocated however its efficacy has not been substantiated

There were no known disturbances in renal function associated with poliomyelitis until it was shown recently in our laboratory that hypercalciuria is accompanied by an increase in the renal excretion of sodium potassium and chloride without alteration of the glomerular filtration rate This abnormality played an important role in the death of one of our patients and it is

quite possible that it may be responsible for some of the chemical disturbances which occur during the acute and early postacute phases of poliomyelitis

BIBLIOGRAPHY

Affeldt J E Personal communication

Albright F and Reifenstein E C Jr The Parathyroid Glands and Metabolic Bone Disease Baltimore Williams & Wilkins 1948

Baker A B Clinical Neurology New York Hoeber 1955

Brady L and Wilson W J Major urologic surgery on poliomyelitis patients confined to respirators J Urol 60 381 1948

Dunning M F and Plum F Personal communication

Gribetz D Sweet A Y Becker A M Levitt M F and Hodes H L Use of anabolic steroids to reduce hypercalcemia and hypercalciuria caused by immobilization in paralytic poliomyelitis A.M.A Am J Dis Child 90 612 1955

Levitt M F Halpern M H., Polimeros D P., Sweet A Y and Gribetz D The effect of abrupt changes in plasma calcium concentrations on renal function and electrolyte excretion in man and monkey J Clin Invest 37 294 1958

Prien E. L and Walker B S Salicylate therapy of recurrent calcium urolithiasis New England J Med 253 446 1955

Rodgers R S Ellwood P M and Ratelle A E *Urological complications of poliomyelitis* J Urol 76 447 1956

Spencer W Personal communication

Whedon G D Deitrick J E. and Shorr E. Modification of effects of immobilization upon metabolic and physiologic functions of normal men by use of an oscillating bed Am J Med 6 684 1949

Wyse D M and Pattie C J Effect of the oscillating bed and tilt table on calcium phosphorus and nitrogen metabolism in paraplegia Am J Med 17 645 1954

DISCUSSION

Dr Neukirch Dr Sweet s paper bears the title of genito-urinary complications in poliomyelitis but is concerned only with the urologic abnormalities However the occurrence of complications involving the genitals should also be mentioned During the acute stage of the disease menstruation disturbances are not infrequent and most—although not all—male chronic respirator patients are suffering from impotency

The poliomyelitis virus per se does not directly affect the urinary organs and the diseases of the urinary tract occur secondarily to a series of complications and specific conditions involved in poliomyelitis which incidentally is not the only disease in which renal calculi occur Not infrequently tuberculosis of the bones and joints will be combined with a calculus formation in the urinary tract a feature which generally is ascribed to the protracted immobilization and the consequent decalcification of the bones

Figures from the epidemic in Copenhagen in 1952 (Table 178) serve to illustrate the incidence of urolithiasis in poliomyelitis Calculi developed in 31 patients including 3 children among a total of 1,258 i e in 2 5 per cent of the cases among 345 respirator patients i e patients requiring artificial ventilation regardless of the duration of this ventilation calculi were demonstrated in 29 i e in 8 4 per cent of the cases Among 26 chronic respirator patients i e patients who more than 12 months after the onset of the disease were still requiring assisted respiration calculi were demonstrated in 13 i e in 50 per cent of the cases Among 31 patients with stone formation recorded in the table 29 had received respiratory assistance however calculi were also demonstrated in the 2 remaining patients although no respiratory paralysis was present As opposed to information given in the paper of Dr Sweet 2 of our chronic respirator patients suffered a development of calculi although their lower extremities were not affected Our material does not give any reliable evidence of relationship between the occurrence of calculi formation or the duration of paralysis of the bladder or the duration of catheterization Several of our patients even those with respiratory impairment in whom catheters were indwelling for several weeks and in whom an additional infection of the urinary tract occurred during the acute stage of the disease failed to give evidence of a stone formation In ? patients apparently the calculi disappeared spontaneously within periods of ? to 3 years

Table 178 Poliomyelitis in Copenhagen 1952—Urinary Complications

	No of Pt's	Calculi Present In	Per Cent
Paralytic cases	1 258	31	5
Respiratory cases	345	29	8 4
Chronic respirator cases	26	13	50 0

Respiratory cases all patients who for a longer or shorter period of time required respiratory assistance

Chronic respiratory cases patients who more than 12 months after the onset of the disease still require respiratory assistance

Two patients with calculus formation—both requiring artificial ventilation—died from nephritis and uremia within 7 months and 3½ years respectively after the onset of the disease

The role of artificial ventilation on calculus formation was only briefly outlined in the introduction In particular the opinion was advocated that calculus formation might become accelerated through hyperventilation investigations indicate that the urinary pH increases the urine thus becoming alkaline during protracted artificial respiration but this feature is not a constant phenomenon The role of artificial ventilation in the pathogenesis of the urinary tract concrements is still remaining obscure

In our material calculi were surgically removed only on one occasion the result was poor and calculi soon reappeared Other investigators found the surgical procedure successful particularly modern surgical and anesthetic

positive correlation between the degree of physical involvement and the emotional difficulties the patient has in adjusting to the disability. The emotional difficulties which are precipitated by the illness and residual paralysis will depend to a large extent upon the degree to which these interfere with the usually successful ways of satisfying emotional needs.

While the illness undoubtedly leaves its emotional scar on the developing personality of the child, the long-term effects of the illness and its possible paralytic residual are less devastating to the infant personality when compared with older patients, because the infant's self-concept usually has not had a chance to develop any significant internal consistence. Thus the person who has an early illness resulting in residual paralysis is frequently able to integrate such residuals into the developing personality structure with a minimum of difficulty. Adaptations are made by such an individual early in life by discovering and cultivating those things in which he is proficient, resulting in adequate ego satisfaction. However, the guilts and anxieties of the parents frequently become a major interference in the otherwise relatively effective adjustment of the ill child. Frequently the parents require the major efforts of the hospital staff in their adjustment to the disabled child.

The age of the patient is likewise of importance in understanding the emotional reaction to illness and the long-term effects upon the personality structure. The infant will react to the physical trauma, the discomfort involved in the diagnostic procedures and the treatment program in a variety of ways—all the way from apparent indifference to stark panic. The infant has basic personality and emotional needs which the home has previously satisfied. These needs are frequently not met in a hospital setting, thus causing bewilderment, fear and generalized panic in the patient. Children frequently find it difficult to understand why they cannot be at home, why their mothers cannot care for them and why they have been abandoned in a strange place with strange people. The emotionally barren hospital environment can be minimized to some extent if the personnel caring for the patient give affection in addition to the physical care. It may be presumed that those infants and children who come from an emotionally secure home environment will experience less threat in the course of their illness and its sequela than those coming from emotionally inadequate homes.

Adolescents who become ill and are left with paralytic involvement have a greater adjustment problem than probably any other age group. In adolescence the self-concept is undergoing further rapid differentiation and refinement. It is in this age period that the personality structure becomes relatively well crystalized, resulting in the establishment of tentative life goals. Relationships with others of the same age become of primary importance, resulting in heightened sensitivity to the approval or disapproval of the group. It has been noted that adolescents who have received their major ego satisfaction through the function of the physique have greater emotional difficulties when left with a residual paralysis than those who depend primarily on nonphysical means for such satisfaction.

Adults react to their illness in various ways, these being determined by psychodynamics similar to those of the younger age groups. In dealing with the emotional reactions of the adult patient, we must try to understand how the illness and its residuals affect his ego needs. Most patients become emotionally disturbed because they anticipate that their illness may make the pre-illness life role impossible. The patient may need to face the problem of how the illness will affect his role as a parent, spouse and sex partner.

There is a difference in the variety and intensity of emotional reactions of poliomyelitis patients. Some of these emotional reactions have been clinically and experimentally studied. One of our investigations has indicated differences with regard to the age of the patient, as well as indicating that male and female patients 7½ years of age and above tend to admit to more emotional turmoil at or soon after onset, while in patients below this age there tends to be a delay of 6 to 8 months in this regard.

There likewise appear to be some differences between males and females in the emotional reaction to varying periods of hospitalization. During the first 6 months after onset most patients express the feeling that they will make a complete recovery. This is probably the only adequate available psychological defense which is able to temper the traumatic significance of

the illness and its paralytic residual We find that our adult male patients have greater defin able anxiety than do the female patients Male patients tend to suppress more of their anxiety than do female patients who may drain off more of their feelings by acting out behavior such as weeping and being unusually demanding of the nursing staff Male patients as a group appear to reach peak emotional tension some where between 4 and 6 months after onset while in the females this tends to appear somewhere between 7 to 9 months

The emotional factors which accompany this illness and the necessary long term hospitaliza tion would have a dampening or inhibiting effect on the effective use of the patient s intel lectual faculties Recently the verbal portion of the Wechsler Adult Intelligence Scale was ad ministered to 93 patients of whom 77 were in patients at the time of testing On a purely descriptive level we found that the average intelligence score of the 46 males tested was 113 and the average score of the 47 females tested was 106 with an over all average score of 109 Two other trends are noted in our sample (1) patients tested soon after admission to the hospi tal tested consistently lower than those who were tested some months or years after onset (2) the patients who were in the younger age groups tested lower than those who were older in age

Another factor which affects the emotional reaction of the patient is the rate of recovery That is patients who make early steady tangible progress in their physical rehabilitation program appear to be less emotionally disturbed than patients who make no progress or whose prog ress is interfered with by secondary medical complications

Further emotional turmoil revolves around the effect the illness has or is felt by the patient to have on the family How the family reacts to and with the patient during the early stages after the onset of the illness has emotional significance for the patient. The patient s illness is an emotional trauma to the whole family Patients of all ages frequently feel that now that they have lost much of their function and may become incapacitated they no longer are lovable or acceptable Such feeling reflects the emo tional significance of the illness to the patient

A number of psychological reactions are clini cally observable during the hospitalization of the poliomyelitic patient During the initial stages of the disease most patients demonstrate feelings of anxiety which are verbalized and sometimes acted out in a variety of ways Some of the anxiety symptoms are related to hyper ventilation which are expressed as feelings of tension and loss of emotional control or even consciousness The expression of anxiety follows a somewhat cyclic course with acute anxiety being followed by a reduction and again an intensification The characteristics of these cycles are of an individual nature and perhaps parallel closely the stages of physical recovery The anxiety which we see in most of the patients is of a reactive type with the bulk of the anxiety being consciously or unconsciously related to the disease and recovery process

Another form of behavior frequently seen in the respiratory poliomyelitic patient can be de scribed as regressive When the majority of a patient s time is taken up with treatment of physical and emotional problems and when he is constantly reminded of the immediate pain involved in the illness it is understandable that his behavior should be less mature and more narcissistic than prior to his illness The patient who has bulbospinal poliomyelitis resulting in muscular paralysis requires 4 hour a-day care during the initial stages of the disease Per haps such intensive care reactivates unconscious patterns of infantile behavior The regressive behavior patterns usually disappear when the patient begins to regain some function making independent movement and breathing possible Patients who are very severely involved ortho pedically and are in the respirator full time frequently exhibit regressive behavior over a period of months and years

Many of the patients become depressed during the course of their hospitalization The paraly sis of the body structure with the subsequent changes which the patient may need to make in his self-concept may cause feelings of de pression The loss of those aspects of the per sonality structure produce feelings similar to grief or sorrow since it is in a sense the death of a part of the pre illness personality Our experience with patients in a reactive depression would indicate that this is usually a healthy sign in that it indicates that the patient is coming to grips with the reality of what the

disease and its residual paralysis means The depression is in a sense a period of mourning for the death of the old self Again patients who make rapid and extensive physical progress in their recovery usually are not as depressed as those who do not make such progress but psychodynamically speaking the patient who makes such progress does not need to be as depressed since the effects of the illness may not necessitate a major personality reorganization The patient who steadfastly insists that his body has not changed or that he has not had poliomyelitis or that he is not paralyzed may not go through a period of depression because he denies that which in reality has happened to him

Hostility is an emotional reaction frequently seen in the bulbospinal poliomyelitic patient. A minimum of hostility is expressed during the initial stages of the disease However as the course of hospitalization continues hostile feelings tend to be expressed The expression of these feelings it is believed indicates that the ego-strength of the person is in the process of redevelopment The expression of negative as well as positive feelings occurs for several reasons The expression of legitimate feelings may help the patient determine how acceptable he is as a person If his feelings though they are of a negative sort are accepted it will make him feel that he as a person is acceptable The first hostile feelings are frequently directed toward the self The patient frequently blames himself for having become ill He may explain it on the basis that he was working too hard or that he had sinned and God is punishing him or in some other way attempt to explain his illness to himself It is probably necessary for many patients to go through a period of self-condemnation as a means of reducing their own guilt feelings about being ill and possibly a burden on others for the rest of their lives It does not take too much ego-strength to express feelings of this sort and since such feelings do not threaten others frequently not too much attention is paid to them

More specific hostile feelings are often expressed These may be directed at the doctor who first saw them This sort of hostility takes more ego-strength and while the validity of what is said may be questioned the significance of the feelings cannot be ignored

Many patients at some time or other express hostile feelings directly to the nurse the therapist or some other member of the hospital staff The patient may condemn the therapist because he has not made a better physical recovery The patient may project some of the responsibility for the illness and residual upon the parents husband or wife

The responsibility of helping the patient with his emotional feelings cannot be assigned to any specific member of the staff but remains a mutual responsibility of the whole team We must accept the feelings of patients in the same way we accept the variety of physical problems which accompany the patient s physical course The Respiratory Center is interested in the whole person and provisions must be made to deal effectively with all problems related to the patient s rehabilitation To facilitate the patient s total rehabilitation the emotional problems must be dealt with effectively

DISCUSSION

PROF DR HOLST What do you know about the mental problems of your fellow men?

Often we will not encumber even our next of kin with all our mental problems—either because they ought not to be disclosed or are so difficult that we are unable to define them Questions of death and God will certainly represent mental problems to most thinking men—yet few of us want to make these questions objects of earnest discussion Most persons do not want to discuss their mental problems if they are ill and personally I never try to probe the minds of dying persons But under less critical circumstances one of the doctor's most important tasks is to obtain the confidence of his patients in order to relieve them if possible of some of those problems which may hurt them and retard their rehabilitation

When very small children are taken ill with polio they have no mental problems because they are not able to create mental problems Unless the individual has reached a certain stage of maturity which is not dependent upon physical age he will not be able to create mental problems even if he is fully able to register impressions of pain

Knowledge of polio is so common that we must expect fright to be the first reaction in a thinking person He will have heard of other patients who have died from the disease or spent years in a respirator or been crippled for the rest of their lives In some cases the fright will be well founded The patient will suffer from respiratory insufficiency and under these circumstances his mind will hardly have room for more than one thought—how to obtain enough air in order to evade suffocation Not to be able to breathe writes one of my patients is the origin of much fright and anguish It is like living in a world where time and air will stand still and tremble Not until respiratory insufficiency has been relieved will consciousness be penetrated by other thoughts These will mainly concern questions of life and death If I live what will be the result? If my hour has come what then? These questions will partly be answered in the course of the first 2 weeks Patients still living after that time may look forward to an indefinite continuation of life

But even now there will not be much occasion for reflection The patient will not have adapted himself to his new and cramped position in the tank cuirass or positive pressure apparatus After a recent tracheotomy he will certainly have troubles from his airways suction must be applied roentgenograms and bronchoscopy will often be necessary He must be tended like a baby Unknown persons will come and go His life will be full of new and mostly painful sensations In fact there will be neither time nor opportunity for reflections beyond the most pressing physical needs

But by and by the patient will realize that there may be a future even for him He will have heard about other cases which have completely recovered His pessimism will give room to optimism and impatience He wants to get out wants to start physical exercise which represents the road to restitution At this time he will have no conception of what will be demanded from him of courage toil and endurance perhaps for the rest of his life

Naturally most of our young patients pity themselves and worry because they are wasting their youth If they have no financial liabilities they will certainly have smaller problems than those who are responsible for a family In these comparatively lucky cases life will be shaped by the degree of invalidity and by impressions from the surroundings

Mental problems will be colored by treatment and improvement Having lost his self-confidence the patient will often energetically oppose any proposal for taking physical exercise which he thinks may hurt him or pain him He will be afraid of breathing with his own muscles or bathing in the Hubbard tank of having his tracheotomy tube removed etc His physical inferiority complex will take a long time to be conquered

His mental problems will be intimately concerned with his fellow patients and the nursing staff His egocentricity will often take the form of jealousy and this may lead to personal antipathies but also can have the opposite effect

the patient will vehemently oppose any change in the nursing staff

A temperamental crippled young lady writes One of the greatest difficulties is how to regain *your self-control when you have lost your ordi* nary means of expression like banging the table and slamming the door It is also very very difficult always to be grateful in a really gracious way

Married people will have more serious mental problems The economic problems will certainly make themselves felt How can the family exist if the breadwinner is taken away or if the working capacity of his wife is so reduced that she must have a substitute? The latter eventu ality is often worse than the former We can generally find financial support for a family *which has lost the breadwinner but it is difficult* to find a substitute for his wife This will cost so much money that the ordinary wage earner cannot afford it and apart from the financial question it is practically impossible to find a capable woman who is willing to tackle a job like that

What about the education of the children if one of the parents is forced to inactivity? If this question has not been given thorough con sideration it certainly *will* be emphasized during idle hours—before sleep sets in Parents will ponder the hundreds of things which they may want to teach the children opportunities and *possibilities which may be lost forever*

What about the healthy partner? Will he or she be able to withstand the stress of the situa tion? Eventually will he or she maintain his or *her feelings toward the patient? Experience has* taught that the patient may indeed be doubtful on this issue

Mental problems will be many and varied according to environment and time Sex and religion ought to be discussed apart

We must remember always that the virus does not destroy either the sexual glands or the brain The crippled patient will keep his hormonal impulses and his daydreams This may lead to a tragic result

In some patients the malady may create a dull apathy but lack of bodily activity may also stimulate mental activity The patient will medi tate upon the meaning of life and the lucky ones may find solace in religion This certainly will be a great help for those who try to lea n that most difficult of arts the art of *resignation*

Care of Patients Severely Stricken by Poliomyelitis

FRIDAY MORNING, JULY 12, 1957

(This Session Convened in the Aula of the University of Geneva)

Chairman

DR HOWARD A RUSK
Institute of Physical Medicine and Rehabilitation
New York

Speakers

DR FRANK S COOKSEY
King's College Hospital
London

PROF DR FRITZ BUCHTHAL
University of Copenhagen
Institute of Neurophysiology
Copenhagen

DR JESSIE WRIGHT
D T Watson School of Physiatrics
Pittsburgh

DR ROBERT L BENNETT
Georgia Warm Springs Foundation
Warm Springs Georgia

DR CARLOS E OTTOLENGHI
Maipu 757
Buenos Aires

DR VERNON NICKEL
Rancho Los Amigos Hospital
Hondo California

Discussants

PROF DR E ADLER
Jerusalem

DR OSWALDO P CAMPOS
Jesus Hospital
Rio de Janeiro

DR GINO COSTA
Hospital Obrero
Lima

DR DON EYLER
Nashville

DR A GROSSIORD
R Poincare Hospital
Garches

DR PAUL R HARRINGTON
Baylor University
College of Medicine
Houston

DR H H JASPER
McGill University
Montreal

DR G A POLLOCK
Princess Margaret Rose Hospital
Edinburgh

DR JAMES W RAE
University of Michigan Medical School
Ann Arbor

DR ARTHUR T RICHARDSON
Kingston Hill
Surrey

DR ROBERT ROAF
Aswestry
Shropshire

DR J W SHARRARD
United Sheffield Hospitals
Royal Infirmary
Sheffield

DR EIVIND THOMASEN
Aarhus Denmark

PROF DR K M WALTHARD
Institute de Physiatrie
Hospital Cantonal
Geneva

Neuromuscular Factors in Rehabilitation After Poliomyelitis

DR FRANK S COOKSEY

Until recently the management of muscles paralyzed by poliomyelitis consisted of general and local rest in the active stage of the disease followed by progressive exercise of the affected muscles during convalescence Rest including the use of rigid splinting often was prolonged because it was thought necessary to prevent weak muscles being fatigued or stretched by the unopposed action of gravity or normal muscles Remedial exercises were graduated carefully to avoid fatigue and localized to weak muscles Trick movements and functional adaptation were discouraged until a prolonged course of remedial exercises had failed to restore normal muscle anatomy

During the past decade or so developments in neurophysiology and clinical practice have led to significant changes in the management of paralytic poliomyelitis At present there is general agreement that rehabilitation after poliomyelitis can be started earlier and carried further than has been customary in the past but there are still controversial views on when remedial exercise should begin and how vigorously it should be pressed Much work has been done and the literature is extensive Even so there is still much to be learned about the nature of muscle fatigue and the factors concerned in the atrophy and recovery of muscles

REST AND ACTIVITY

The long-established value of general and local rest in limiting the spread and aiding the resolution of any infection is still undisputed In poliomyelitis, in particular there is evidence that rest in bed in the prodromal stage and while the infection is still spreading is a significant fa tor in limiting the incidence of paralysis Nevertheless like most therapeutic agents rest has adverse side-effects about whi h much more has been learned in recent years leading to important changes in many aspects of clinical practice For instance early ambulation after surgical operations and in the management of the degenerative disorders of the elderly can be cited as examples

Mainly due to the advent of antibiotics it has become possible to restrict the use of rest in the presence of infection This has emphasized the adverse effects of rest which were formerly dismissed as being of secondary importance to the control of infection In the absence of an antibioti for poliomyelitis rest continues to be of major importance until this infection has subsided Nevertheless even in the acute stage a balance can be struck between the beneficial and adverse effects of rest

Many workers have drawn attention to the severe wasting which occurs in normal muscles when they are immobilized especially when immobilized in the shortened position Similar wasting occurs in muscles involved in peripheral nerve injuries and in poliomyelitis This wasting was attributed to reduction in blood flow consequent upon inactivity but it now seems to be recognized that the maintenance of tone in normal muscles is mainly dependent upon the proprioceptive stimuli developed in the muscle spindles during movement Wasting and reduction in blood flow follow when these stimuli are reduced by immobilization or by interruption of the afferent or efferent neurons In experimental animals the effect of immobilizing normal muscles in the stretched or shortened position is to cause as much wasting as when the anterior or posterior roots are divided although in recent work it was found that the blood flow had actually increased

A feature of poliomyelitis is that stiffness and contracture of muscles may set in early and insidiously lead to intractable deformities Immobilization tends to increase the contractures while passive movement helps to prevent or overcome them

Less is known about the effect of rest and activity on the central nervous system Some workers hold that the transmission of stimuli by synapses when inflamed by the virus is

detrimental to them Others point out that synapses are so sensitive to anoxia caused by inflammatory edema that they do not respond to stimulation until recovery is well advanced when no harm ensues Nerve cells which survive the infection appear to recover in a few weeks so that even if rest is necessary in the acute stage it need not extend beyond early convalescence

Based on the foregoing the present trend in the management of the acute stage is to rely on general rest in bed to combat the infection and to employ passive movements from the onset in order to maintain some proprioceptive stimulation to aid the circulation in the muscles and to prevent contractures Gentle stretching of weak or paralyzed muscles during passive or active assisted movement is now thought to be beneficial rather than the reverse At rest muscles are maintained as far as possible in the neutral position by means of pillows or sandbags and the use of rigid splints or plaster casts is avoided except when necessary to oppose the action of gravity as for example with opponens pollicis

When the infection has subsided and convalescence has been established for some 3 or 4 weeks unaffected muscles are exercised to prevent further wasting and weak muscles started on graduated exercises At this stage two points need to be considered First the effect of fatigue on weak muscles Second whether to exercise individual weak muscles or to develop them together with other affected or normal muscles in their functional movement patterns

One of the controversial problems is the effect of fatigue on muscles recovering from poliomyelitis In the past fatigue was thought to b harmful and great care was taken to avoid it Recently Ritchie Russell has stated that as fatigue is the physiologic stimulus to muscle hypertrophy remedial exercises should be graded to produce it if the maximum benefit is to be obtained In between those who say avoid fatigue and those who say push muscles to fatigue are those who hold that fatigue in moderation does no harm but that it is not necessary to fatigue muscles in order to increase their strength

Fatigue may be due to muscular effort exceeding the available blood supply so that metabolites accumulate faster than they can be removed However the commonest cause of fatigue in muscles is fatigue in the corresponding synapses of the nervous system This applies both to synapses concerned in the direct motor supply to a particular muscle and in the associated pathways which co-ordinate its action in a functional movement pattern Synapses are very sensitive to disuse and respond rapidly to exercise There is no convincing evidence that synapses are harmed by being fatigued

There is a difference between physiologic fatigue and excessive exercise leading to muscle exhaustion The latter may cause marked swelling followed by ischemia and necrosis in muscles which are confined by aponeurosis such as the anterior tibial group This effect is easily produced in partially denervated muscles and care must be taken to avoid it

THE RE EDUCATION OF WEAK MUSCLES

In the early stages of convalescence there is spontaneous recovery of those motor units whose anterior horn cells have been put out of action temporarily but not destroyed While the recovery of function is spontaneous up to a point the disuse atrophy in the muscles and in the synapses of the central nervous system will persist until reversed by progressive activity That is to say rehabilitation is necessary even for muscles which recover from an attack of poliomyelitis if full function is to be restored without undue delay One so often sees patients who make good recovery with practically no weak muscles but then everyone thinks there is no need to do anything about rehabilitating them Of course there is the same need to rehabilitate those patients as anyone else who sustains severe illness

Recent work on the integrated activity of the central nervous system and the importance of the proprioceptors in the muscle spindles as the focal points in the control of movement have led to the development of new technics of re education through functional movement patterns in preference to training individual weak muscles This important advance in kinesiology has greatly facilitated rehabilitation after poliomyelitis as well as in other disorders of the locomotor system

Nevertheless in poliomyelitis in particular there is still a place for the training of individual muscles especially in the early stages Muscles

which lose motor units through the destruction of anterior horn cells have to compensate by reorientation and hypertrophy of the surviving motor units within the affected muscles There upon the central nervous system will tend either to modify the movement patterns in which the affected muscles are concerned so as to make the best functional use of what remains or to substitute other normal muscles if available for the weak ones which will then atrophy from dis use The weaker the muscles the greater will be the tendency to substitution and conse quently the lesser the likelihood of the affected muscles being strengthened by being used Therefore it is essential to exercise individual weak muscles to obtain the maximum hyper trophy of surviving muscle fibers in order to avoid unnecessary substitution

However the training of individual weak muscles will not restore functional activity as a whole It a weak muscle is exercised in isola tion fatigue at first sets in rapidly but dimin ishes with facilitation Yet if the muscle is now exercised in its normal movement pattern it will again fatigue because of lack of facilitation in the mechanism of associated movement Therefore the training of individual weak mus cles must be combined with re-education through functional movement patterns

Exercising muscles to the point of fatigue against increasing loads improves their power i e the maximum tension which they can exert Facilitation of the synapses in the central nervous system through practice increases the speed co-ordination and endurance of muscle action Accordingly the achievement of full re habilitation without undue delay necessitates a program of remedial exercises and occupa tional therapy starting as early as possible and calling for increasing effort by the patient for longer periods each day until the maximum re sponse has been obtained That is to say one must provide decreasing effort against load and in creasing practice to obtain co-ordination and endurance and speed A recent study of two groups of cases subjected to intensive rehabilita tion, one starting at 4 weeks from the onset and the other at 8 weeks has shown a similar degree of improvement in the affected muscles and no untoward effects in the group which started at 4 weeks

Poliomyelitis is an extremely variable disorder and clinical judgment must be relied upon in the management of individual cases Nevertheless the recent work does seem to suggest that in general rehabilitation after paralytic poliomye litis can safely commence 1 month from the onset and be pressed more vigorously than has been customary in the past

BIBLIOGRAPHY

Bowden R E M Comparative study of rate of atrophy of skeletal muscle following anterior ramisection and acute anterior poliomyelitis in rhesus monkey Bull Johns Hopkins Hosp 89 153 1951

Darcus H D Discussion on an evaluation of the methods of increasing muscle strength Proc Roy Soc Med 49 999 1956

Hellebrandt F A Kinesthetic awareness in motor learning Cerebral Palsy Review vol 14 nos 5 and 6 1953

Howe H A and Bodian D Neural Mecha nisms in Poliomyelitis New York Common wealth Fund 1942

Imig C J Randall F and Hines H M Effect of immobilisation on muscular atrophy and blood flow Arch Phys Med 34 296 1953

Russell W R Poliomyelitis The pre paralytic stage and the effect of physical activity on the severity of paralysis Brit M J 2 1073 1947

——— Paralytic poliomyelitis the early symp toms and the effect of physical activity on the course of the disease Brit M J 1 465 1949

——— Poliomyelitis ed 2 London Arnold 1956

Wynn Parry, C B Personal communication 1957

Young J Z Effects of use and disuse on nerve and muscle Lancet 2 109 1946

DISCUSSION

Dr Sharrard I would like to endorse much of what Dr Cooksey has said in his careful resume of current trends and thoughts on the treatment of paralyzed patients

During the past 50 years all manner of treatments have been used many of them based on accepted custom ignorance prejudice or misapplication of facts rather than on an appreciation of the pathology physiology and clinical progress of the disease What Dr Cooksey has said this morning shows that we are now much more appreciative of these things

General rest during the acute stage is vital to minimize the spread and activity of the virus in fact 1 or 2 weeks of bed rest may not be enough because a relapse with serious consequences can occur as late as the third week Vigorous active exercises during the first 3 weeks also can cause further spread of the paralysis which should be a warning to those who are overenthusiastic that there is a lower limit to the time at which an active program should begin

However passive joint movements and muscle stretching are safe and are essential to prevent contractures Weak or paralyzed muscles are no more susceptible to stretching than normal muscles On the contrary excessive relaxation of muscles is likely to discourage recovery as the following results show Of 81 completely paralyzed muscles treated on abduction frames for periods up to 2 years 17 recovered of 93 completely paralyzed muscles that were treated on no abduction frames 35 recovered

TREATMENT IN THE CONVALESCENT STAGE

It is not appreciated generally that spontaneous recovery of intact but inactive motor units though most definite during the first 6 weeks may still continue up to the fourth month The motor cells in this photograph (Fig 334) were all that remained of the nucleus of the quadriceps muscle all the cells there were in a patient who died suddenly at the end of the fourth month He had been receiving active physiotherapy treatment yet the quadriceps had only started to show a flicker of contraction 2 weeks before he died in other words those cells had been in abeyance for 4 months Note the vascularity and cellular infiltration of the supporting tissues 6 months after the disease Edema which is a prominent feature does not finally subside until the eighteenth month

While it is doubtful whether exercises or the lack of them make any difference to the intrinsic recovery of the neuron I agree with Dr Cooksey that the resumption of muscle action needs active effort and that this is probably better achieved by the training of individual muscles or muscle groups than by the use of functional movement patterns in the early weeks This program of exercise can well commence at the fourth week



Fig 334 Gray matter of spinal cord 4 months after acute anterior poliomyelitis Two motor nerve cells constitute all that remains of the nucleus of the quadriceps muscle The muscle showed clinical contraction before death and a streak of innervated fibers was found in the muscle at postmortem Note the vascularity and cellular infiltration (Proceedings of the Second International Congress of Neuropathology London 1955)

After the second or third month it is logical that training in movement patterns should be introduced and gradually predominate over individual muscle exercises in preparation for a return to functional activity

I mention electrical stimulation of muscle or nerve only to condemn it Spectacular as it may be to a patient to see his paralyzed muscles acting under its influence there is no physiologic basis for its use it wastes time discourages voluntary activity and in clinical trials does not accelerate or increase the amount of recovery

Concerning fatigue and exercises while I accept that fatigue defined as a falling off in power of a muscle that is being exercised is not harmful should it occur I cannot believe that fatigue should be the aim of treatment

An intensive exercise program too is impracticable in a patient with many weak muscle groups If as has been suggested a muscle group should be exercised for 5 minutes every hour then it is only possible to treat 9 or 10 muscles or muscle groups in this way

However if a muscle is to become more efficient it must be made to work hard In my experience a regimen in which muscles are exercised up to the point of fatigue and all weak muscles are given an equal amount of attention during the day achieves as much as a more intensive method In support of this I show the results of an analysis of muscle power (Table 179) after the completion of such a program just to demonstrate that with such a program

TABLE 179 THE RELATIONSHIP BETWEEN MUSCLE POWER AND RESIDUAL MOTOR CELLS IN THE SPINAL CORD

MUSCLE POWER (M R C SCALE)	PERCENTAGE OF RESIDUAL MOTOR CELLS
0	0–2
1	2–3
2	3–5
3	5–10
4	10–20
4	20–40
5	over 40

These figures were obtained by counting all the remaining motor cells in each muscle nucleus from complete serial sections of the spinal cord and comparing them with the known clinical power of the muscle before death

muscles which are grade 3 need only to have 5 to 10 per cent of residual motor cells and a muscle which is grade 5 that is normal may lose as much as 60 per cent of its nerve cells and still work as a clinically normal muscle

PROF DR WALTHARD First I would congratulate Dr Cooksey for his excellent communication I quite agree with his view that it is indispensable if one does not want to lose or waste precious time or even jeopardize the future of the poliomyelitic patient to start with muscular exercise either by passive movements wherever possible or by active kinesiotherapy as quickly as possible after the acute phase of the disease In this direction I even go further than my colleague There are indeed cases in which the general state of health has not been seriously affected I have adopted as a working principle the procedure of undertaking muscular re-education as soon as the patient can actively collaborate in the exercise

When the patient has returned to what I call the normal vegetated balance that is if he no longer has any fever and he sleeps and eats well then he must not be considered a diseased person or an unhealthy person but an invalid with good general health Disease and invalidity are not synonymous If the person is sick then every effort must be avoided because a sick person needs all his reserves of strength to struggle against the disease whereas the invalid is an individual in whom the state of health is re established or recovered but who still has lesions in this case of the locomotor nervous system and therefore of the muscular system

In regard to the intensity of physical exercise it might not be superfluous to remind that the therapist must take into account the general state of health of the patient Just as in sports training must be undertaken carefully and adapted to each case If the patient has had to remain in bed and with complete rest we must not now go to the other extreme and overburden the recovering patient by a too intensified training It is important in this respect to observe for instance the muscular reactions after repeated stimulation with galvanic current As the electric stimulation sessions are prolonged contraction of a paralyzed muscle group will decrease in correlation with the intensity of the applied current In other words muscular or synaptic fatigue

turns up much more quickly than in normal muscles One should not prolong electric stimulation sessions beyond approximately 10 minutes

What has been observed during electric treatment also can be seen in medical gymnastics sessions We should not ask the patient to make too much of an effort at the beginning of the treatment The readaptation can be continued over years but as with other persons the poliomyelitic person has a need for holidays Therefore the treatment should be varied and differentiated as much as possible and if pursued over long consecutive months should be interrupted completely and frequently We have been able to observe that an interruption of every treatment during the holidays under another climate for instance in the mountains of Switzerland helps the patient considerably After a change of environment and of climate the paralyzed muscles had spontaneously recovered some vigor and the poliomyelitic patient could much more easily tackle his medical gymnastics The holidays the stay in the higher mountains for instance made it possible for him to accumulate new reserves of strength reserves which he needed for future readaptation and from which the neuromuscular system had benefited in the first place

Prognostic Implications of Electromyography in Poliomyelitis

PROF DR FRITZ BUCHTHAL

This report reviews the evidence as to the extent to which electromyography in the early stages of poliomyelitis allows the final degree of paresis to be predicted Such a study requires investigation both by electromyography and by grading of muscle force within the first month after the onset of the disease and reinvestigation, if possible repeatedly but in any case 1 year later

What aspects of the electromyographic pattern are to be considered in such a comparative study? First is the general pattern of electrical activity of the muscles during maximum voluntary effort This ranges from the so-called interference pattern of normal muscle with the simultaneous activity of numerous motor units to the pattern seen in severe neurogenic paresis where the activity recorded is of single motor units This range of electromyographic patterns agrees fairly well with the grade of muscle force as estimated by experienced physiotherapists (Fig 335) The abscissa is muscle grading and the ordinate is a pattern of electromyographies the lowest single oscillations and above interference patterns indicating the activity of many motor units At 1 year after the onset of the disease the degree of electrical activity corresponds to a relatively higher degree of muscle force than at the onset Evidence exists that this discrepancy is not due solely to the inability of acutely involved painful muscles to work at their potential maximum Rather it may be due to an increase in later stages of the disease in the size of the motor units by secondary branching of the nerve fibers to include denervated muscle fibers

Then there are the characteristics of the single motor unit potential duration amplitude and wave form Finally there is synchronism the interrelation of discharges in different regions of the same muscle to a degree exceeding that in a corresponding normal muscle

In addition to these characteristics of the electromyogram during voluntary effort there is the electromyographic picture at rest There is no electrical activity in relaxed normal muscle but 2 to 4 weeks after partial or total denervation spontaneous discharges occur which have a much shorter duration than the ordinary motor unit potentials

Comparative studies of the electromyogram and muscle force in paretic muscle during the acute stages of poliomyelitis have been carried out in Copenhagen beginning in 1942 and this series of patients and 2 subsequent ones have been in agreement that there are very definite correlations between the early electromyographic findings and the final degree of muscle paresis The most recent series of patients was the largest and the most carefully controlled 45 children were studied at the Boston Children's Hospital in 1952 and were re-examined in 1953 The findings are shown briefly in Table 180

To estimate the prognostic value of action potential analysis the patients were grouped according to the degree of initial paresis and of

TABLE 180 GROUPING OF PATIENTS

Group	State of Muscle When Examined		Recovery
	At Onset	At 1 Year	
R⁻	Zero to fair	Zero to fair	Zero to slight
A	Fair to good⁺	Fair to good⁺	Zero to slight
R⁺	Zero to fair	Fair⁺ to normal	Large
N	Good to normal	Good to normal	Zero to slight

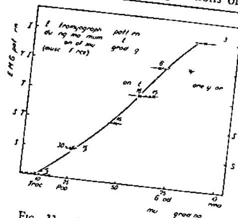


FIG 335 Electromyographic patterns during maximum effort as a function of muscle grading

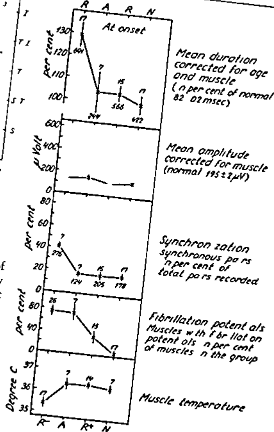


FIG 336 Patients grouped according to the degree of initial paresis and of recovery from it 1 year later

covery from it one year later On account of shortage of time I beg you to pay attention only to the group nominated R— a group without recovery and the equally paretic group R+ with recovery 1 year after the onset of the disease That is to say that the 2 groups had muscles which were equally paretic at the onset but the plus group showed greater improvement in the course of 1 year

The main results are shown in Figure 336 and again on account of shortage of time I beg you to look only on the left column and again on the group R— and the group R+

Let us begin with action potential duration The mean action potential duration obtained at a constant intramuscular temperature corrected for the age of the patient and for the muscle investigated was in the acute stage of the disease 30 per cent increased in severely affected muscles without signs of recovery 1 year later (group R—) Severely paretic muscles which improved greatly in the course of 1 year had normal values of mean action potential duration In paretic muscle of all groups the incidence of polyphasic potentials was double that in normal muscle without regard to the initial degree of involvement or the final recovery The increase in polyphasic potentials thus cannot account for the increase in mean potential duration in the R— group Mean action potential amplitude was not correlated with recovery

The incidence of synchronous activity between different leads in the same muscle was clearly correlated with the degree of recovery Synchronism occurred in 40 per cent of paired leads in severely paretic muscle without recovery (R—) against 20 per cent in the other groups and in normal controls In the majority of the cases synchronization occurred within 10 days after the appearance of the paresis in some cases as early as 1 day after the clinical manifestation of paresis A synchronization still present after the twentieth day persisted more than 6 months or 1 year after the paresis and was found in muscles with no recovery

In paretic muscles with no recovery spontaneous low voltage activity of short duration (fibrillation potentials) occurs in a much higher percentage than in muscles with good recovery The difference was 75 per cent against 33 per

cent. There was no spontaneous motor unit activity at rest in the cases which we have seen There is no evidence of spasm in affected muscles either initially or after recovery

An incidental finding (as indicated in the last column) was that the intramuscular temperature was correlated to the degree of involvement and recovery in that severely affected muscles without recovery showed a significant decrease as compared in temperature about 1 degree as compared with other groups and normals

The finding of a correlation between muscle action potential duration and synchronism in the acute stage of poliomyelitis on the one hand and degree of recovery as determined by muscle force grading on the other indicates that the eventual outcome of the muscle paresis is largely determined by the initial disease process Early electromyography thus may provide a guide as to which muscle will and which will not benefit by physical therapy directed to those muscles

As to the cause of the electromyographic changes in the paretic muscles in the acute stage of poliomyelitis the electromyographic pattern at maximum effort is readily explained by the loss of many motor units which in the later stages corresponds to the histologic picture of scattered islands of normal tissue on the back ground of degenerated muscle Recently it has been shown that the duration of motor unit potentials in normal muscle depends on the spatial distribution of the innervation zone of those muscle fibers of the motor unit which contribute to the motor unit potential Thus it is most likely that the increase in action potential duration in muscles paretic from poliomyelitis is due to an unusually wide innervation zone this might occur if the motor unit undergoes an enlargement such as could be brought about by the simultaneous firing of more than one of the surviving ganglion cells A simultaneous firing would not only give rise to a pseudo-enlargement of the motor unit but also would evidence itself as a synchrony in different regions of the muscles Recent multi-electrode studies on the area occupied by the fibers of a motor unit have shown that this may be up to four times as large in severely affected polio paretic muscles as in normal muscle the area exceeding even that of the largest motor units in normal muscle Thus survival of the largest motor units cannot account for the average increase in potential duration in the early stage of the disease Another possible cause of the increase in action potential duration i e a decrease in the velocity with which the action potential is propagated over the individual fibers has been invalidated by recent measurements of the propagation velocity over severely affected muscles

BIBLIOGRAPHY

Bowden R E M and Gutmann E Denervation and re innervation of human voluntary muscle Brain 67 273 1944

Buchthal F Guld C and Rosenfalck P Innervation zone and propagation velocity in human muscle Acta physiol scandinav 35 174 1955

——— Multielectrode study of the territory of a motor unit Acta physiol scandinav 38 331 1957

Buchthal F and Honcke P Electromyographical investigation of patients suffering from poliomyelitis ant.ac. up to six months after the acute stage of the disease Acta med scandinav 116 148 1944

Hertz H Madsen A and Buchthal F Prognostic implications of electromyography in acute anterior poliomyelitis J Bone & Joint Surg 36A 902 1954

Huddleston O L and Golseth J G Electromyographic studies of paralyzed and paretic muscles in anterior poliomyelitis Arch Phys Med 29 92 1948

Pinelli P and Buchthal F Duration amplitude and shape of muscle action potentials in poliomyelitis Electroencephalog & Clin Neurophysiol 3 497 1951

Wohlfart G Aktuelle Probleme der Muskelpathologie Deutsche Ztschr Nervenh 113 426 1955

DR JASPER Our experience with the use of electromyograph for prognosis in poliomyelitis is based largely upon examinations carried out with Mrs Gwen Ballem of 50 patients stricken during the epidemic in Montreal in 1946 We examined 35 of these during the first 2 or 3 weeks following the onset of their illness and followed 25 with re-examinations from 9 months to more than 1 year

Our results insofar as we have repeated the simple procedures have been in general confirmation of those presented by Dr Buchthal with a few additional findings which I present for discussion

I was surprised to hear that Dr Buchthal and his associates did not find any evidence for spasm or spontaneous motor unit activity during the initial stages of the disease In a number of our patients spasm was a prominent feature of the disease during the first week or 10 days In some this was of sufficient severity to threaten flexion deformities of the limbs and it was painful It was a reflex spasm since it could be made to disappear almost entirely in most cases by posturing the limb sufficiently to eliminate any stretch on the muscles In 2 patients with the assistance of an experienced anesthetist the spasm was abolished completely by blocking of the dorsal roots with 5 per cent procaine This produced a sensory anesthesia of the affected limbs without a motor paralysis The muscles relaxed completely even with full extension of the limbs and voluntary movement was preserved The involved muscles were then silent at rest with no fibrillation or motor unit discharge except on voluntary movement However although the presence of spasm was of importance during the early stages of the management of these cases it did not seem to imply a poor prognosis for eventual paralysis unless it was succeeded by a flaccid paralysis and fibrillation or permanent electrical silence in the electromyogram

Another observation not mentioned by Dr Buchthal caused considerable concern This is the electrically silent muscle showing no fibrillation or motor unit activity even during maximum voluntary effort We have found such muscles in some patients even during the acute stages of the disease with no recovery and severe atrophy found on re-examination 9 months or 1 year later One such patient was examined on 4 occasions during a year and we were never able to detect any electrical activity in the completely paralyzed quadriceps from the beginning Neither were we able to produce a muscle contraction by direct electrical stimulation Such findings though not common carried with them the poorest prognosis more severe than the fibrillating muscle We were under the impression that such results must imply an involvement of the muscle tissue itself by the disease process since no such results have been seen in extensive studies of traumatic lesions of peripheral nerves except when a severe ischemia of the muscle has been added as well

Interpretation of an electrically silent muscle is not always easy unless combined with studies of indirect and direct electrical stimulation We have found in all cases that the interpretation of electromyographic results is greatly improved if combined with the results of electrical stimulation In a few cases we have found an electrically silent muscle which responded well to electrical stimulation and to reflex excitation and yet the patient was unable to move it voluntarily Hysterical paralysis is always considered in such cases but in a few instances we felt that there was a genuine organic difficulty in the voluntary innervation of muscles not necessarily involving the peripheral muscular system

Also there is the interpretation of the polyphasic action potential of long duration There are those which occur early in the disease similar to those seen in progressive neuromuscular disease like progressive muscular atrophy which must have a different interpretation than those occurring late In the later stages the interpretation of Dr Buchthal seems reasonable that there is extensive terminal branching of fibers causing enlargement of the motor unit and we have seen this enlargement to be very great indeed in certain cases It would seem that any treatment which would increase this natural tendency

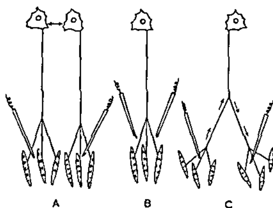


FIG 337 Three types of synchronism of motor unit activity

should be quite beneficial in muscles which are only partially paralyzed

DR RICHARDSON In his paper Professor Buchthal has described the electromyographic changes of muscles in the early stages of acute poliomyelitis and related them to the final degree of paresis In detail the changes were first a 30 per cent increase in the mean duration of the motor unit action potentials second an increase in the synchronism of motor unit activity third an increased incidence of spontaneous fibrillation potentials

Concerning the increased incidence of spontaneous fibrillation potentials he found first they occurred in 75 per cent of the poor recovery group compared with 33 per cent of the good recovery group This is to be expected because the presence of fibrillation potentials is an index of lower motor neuron degeneration In poliomyelitis the fact that this degeneration arises from irreversible motor cell damage must mean that regeneration of the low motor neuron is permanent Hence finding fibrillation potentials must be of significance However the trouble is that the onset of fibrillation in poliomyelitis as indeed in any other cases of low motor neuron degeneration is delayed for 3 or 4 weeks Nerve conduction measurements are lost in a matter of hours after the degeneration For this reason particularly Brooks in England and most of us now use nerve conduction measurements in the prognosis of treating muscles in poliomyelitis

Returning to the question of the increased duration of motor unit potentials and synchronism of motor unit activity Professor Buchthal is going to reopen I am sure purposely the controversy which has now been waiting for 14 years This question of synchronism besides the fact that it was first introduced by Clemmesen and Buchthal is a most interesting phenomenon and of course it is not confined to poliomyelitis it occurs in other co lesions

When they first introduced the term synchronism of motor unit activity their suggestion was that two motor units beat in unison due to spread of impulses in the damaged cord This is illustrated in A of Figure 337 In A you see two motor units two anterior horn cells at the top and according to Buchthal and Clemmesen there is a spread of impulses across the anterior horn cells with the two beating in unison Some support for this comes from the fact that one can obtain synchronism in strychnine poisoning and other co lesions However Denny Brown later in 1949 put forth another theory which perhaps has been more generally accepted This I have illustrated in B with two needle electrodes apparently picking up the same motor unit potential There is much evidence in support of that as Denny Brown s own work was based on measuring the mechanical response and the electrical response at the same time Kugelberg and Taverner appeared to complete this study by showing that synchronism could occur on peripheral stimulation of the nerve therefore virtually excluding the idea that it was due to a spread of impulses in the cord However as Professor Buchthal has pointed out repeatedly there is no doubt that at times one can localize two motor potential sources With this in mind Burns and I suggested the third theory C and that was that the residual motor units in poliomyelitis were often branched Our evidence for that was based on the fact that we sent a stimulus along one needle electrode and got an excellent reflex at about 4 msec It does seem a most likely explanation of synchronization of motor unit activity as selective destruction of anterior horn cells leaving the large branched units However Professor Buchthal s suggestion indicates first

that this cannot be served because the residual motor potentials are too great to be accounted for by survival of the large ones and second that measurements of the velocity of the fiber action potential are not compatible with this theory and will I think reopen this whole subject again

Therefore in conclusion whatever the explanation of this mysterious phenomenon of synchronization it is clearly of prognostic importance as is the instance of fibrillation potential As to its mechanism we need much more study before we can decide

BIBLIOGRAPHY

Bremer F (cited by Fulton J F) Physiology of the Nervous System ed 3 New York Oxford 1951

Buchthal F and Clemmesen S Acta psychiat Kbh 18 377 1943

Denny Brown D Arch Neurol & Psychiat 61 99 1949

Kugelberg E and Taverner D Electroencephalog & Clin Neurophysiol 2 125 1950

Richardson A T St Thomas Hosp Rep 7 164 1951

Wohlfart C and Swank R L Arch Neurol & Psychiat 46 783 1941

Physical Medicine and Orthopedics in Treatment of Patients with Respiratory Paralysis

DR JESSIE WRIGHT

In all phases of care of poliomyelitis psychological and emotional factors are of great importance especially in treatment of patients with respiratory paralysis Anxiety and neuromuscular tension may be obstacles to a sense of ease and confidence which makes breathing easier and more rhythmic no matter what degree of paralysis is affecting respiration Tension may reflect a feeling of insecurity and besides blocking neuromuscular mechanisms may lead to restriction of blood flow and unnecessary shortening of tissues All this may contribute to discomfort or to a tendency to deformity as well as poor mechanics in joints and muscles which receive most of the blame for disability Anxiety and tension also may interfere with co-operation by distracting the patient from a favorable program which has been prescribed A person does not need to be paralyzed to realize how emotion alters breathing These factors added to actual paralysis may place respiration at a greater disadvantage than is necessary

All professional personnel from the onset of illness should show an interest in the patient s personal worth and attainment This is particularly important in a patient recovering from impaired breathing Physical treatment or prevention of disability and deformity should not form the central theme of the patient s existence Educational or prevocational guidance should proceed with equal pace as the general condition permits The physician the nurse the medical social worker and the psychologist as well as all other professional personnel can help the patient and family with these problems which may influence the progress toward recovery The neuropsychiatrist may be one of the consultants for special disturbances But all those having contact with the patient should be oriented to the multiple needs during convalescence

Introducing re-education procedures early in convalescence helps patients to make the transition from necessary ministrations during the acute stage toward greater independence and practical goals Otherwise patients who have had respiratory distress may become tyrants who resist weaning from mechanical aids

As soon as the temperature has been normal for 48 hours or when the general condition permits the costovertebral and facet joints should be mobilized and kept free during convalescence Most often these joints are overlooked while the joints of the extremities are moved in a desirable way from the early days of illness Freedom of the costovertebral and facet joints is essential to easy migration of the ribs in breathing

Not only are mobile costovertebral and facet joints essential to optimal breathing but such movement favors proprioception in the joints and muscles of respiration helps blood flow moves metabolic products and limits the discomfort and aching that result from inactivity Adequate working of the chest bellows helps to blow off carbon dioxide and to take on oxygen necessary to metabolism in all organs and tissues

When the physician prescribes exercises taking advantage of the momentum of the rocking bed he asks the physical therapist to use an active assisted range of motion for the costovertebral and facet joints of the trunks as well as for the extremities and neck

When the head of the bed comes up the patient inhales Then he rests for one or more cycles of rocking repeating the exercise with every second or third excursion of the bed Next the patient pushes his head backward against the bed chin level at the same time arching the upper back and pulling the shoulder blades together while inhaling as the head of the bed rises During expiration the physical therapist compresses the lower chest as the head



Fig 338 Applying compress on to the thorax As the head of the rapidly rocking bed goes down ard the physical therapist compresses the lower thorax helping the patient to exhale and mo ing the costovertebral joints



Fig 339 Releasing compress on to the thorax As the head of the rapidly rocking bed rises the physical therapist releases the lower ribs helping to mobilize the costovertebral joints as the patient inhales

of the bed goes down helping exhalation (Figs 338 and 339) Both of these maneuvers mobilize costovertebral joints Next the patient makes active effort as the therapist twists the thorax for mobilization of the costovertebral and facet joints During this maneuver the physical therapist reaches under the thorax with one hand around each side and holds the shoulder blades toward each other to protect the interscapular groups from stretching as will be seen in Figure 340

The visceral shift during the two phases of movement of the bed not only moves the diaphragm but also helps to mobilize the costovertebral joints If the diaphragm is better than the intercostals further migration of the ribs may be attained by using a rocking bed with chest bellows and shell synchronized with the rocking This may be seen in the exhibits The chest shell should be placed high on the trunk to permit as much excursion of the upper thorax as possible

Posture of the head in relation to the thorax will influence breathing Whether or not the patient is in a tank lung a chest respirator is receiving internal positive pressure breathing or is on a rapidly rocking bed the effectiveness of any of these aids will be influenced by the relative position of the head on the neck and the neck on the trunk The patient's position in bed should be comparable with standing tall with the back of the head high and the chin level which elevates the ribs

Efficiency of breathing and circulation is affected by the tone of the abdominal muscles The pull of the tank respirator may stretch weak abdominals If the chest respirator is not fitted carefully expansion in the epigastrium may be exaggerated while movement of the ribs may not be attained because the shell has been placed too low on the trunk

On the rocking bed if the abdominals are weak as the head of the bed rises one sees from a side view that the visceral structures migrate going beyond the desirable downward pull on the diaphragm to cause a protrusion of the lower abdominal wall In order to encourage a erage diaphragmatic shift protect the abdominals from stretching and favor circulation a support should be given to the trunk from over the pelvis as far up as the dependent ribs The part below the umbilicus should be fastened firmly with graduated support looser above so as not to limit excursion of the diaphragm In order to prevent riding upward of the support a many tailed scultetus binder may be used carrying the lowest strip on each side under the groin as a perineal strap (Fig 341)



Fig 340 The phys cal therap st mob l zes the costo ertebral and facet jo nts to make breath ng eas er

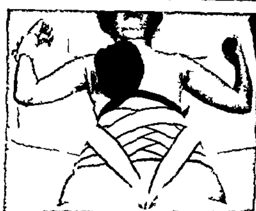


Fig 341 A sandbag l m ts motion of the s de of the chest that expands be ter wh le the pat ent concentrates on inhal ng mo e *on the l m ted s de as the head of the* rap dly rock ng bed rises A many ta led scultetus b nder holds the lower abdomen to pre ent protrus on of a weak abdom nal wall as the head of the bed r ses

It the d aphragm is good and the ntercostals poor it is part cularly mportant to use a support for the lo ver abdomen or else the upper chest actually may be depressed during expan s on n the ep gastrium When the pat ent is *mpro ed enough to ha e the bed s opped for* meals and the ba k and knee rests are cranked into a sem s tt ng pos t on the blood pressure may drop and the pat ent may feel fa nt espe c ally f the abdom nals are weak When a trans t on s made to a more erect s tt ng pos t on in bed or on the s de of it the support of the lo ver *abdomen should be firmer and in slender pa* t ents a lo ver abdom nal pad or inflatable mold placed to l m t p otrus on of the lo er abdomen Th s also guards aga nst fall n blood pressure wh ch may be enough to cause fa nt ng and further l m tat on of breath ng

Much of the st ffness seen n sp nal jo nts and extrem t es *can be l m ted or p e en ed if the* phys cian seeing the pat ent early n llness has the interest and cou age of h s con ict ons in l m t ng the use of tank lungs and in progress ng to internal pos t e pressure breath ng carefully regulated or by us ng the cuirass shell w th the rap dly rocking bed Under the bu den of an *o e whelm ng number of pat ents dur ng sea* sonal outbreaks or ep dem cs t may be hard to be at hand to change the prescr pt on for resp ratory a ds to meet the trans t ons in the cond t on of pat ents But only n th s way w ll unnecessary impa rment of chest or extremi y mo on be pre en ed circulat on *facil ta ed and* tolerance de eloped for subsequent phys cal and occupat onal therapy wh ch is d rected toward reco ery as complete as the or g nal nsult to the ner ous system perm ts Of course tolerance for any of the mentioned act t es w ll depend on ma nta n ng the v tal functions pre ously co ered n th s conference by the *phys ologi* ped atr c ans and intern sts who are concerned w th *such early care wh ch may be l fesa ng*

STEPS IN REHABILITATION

Deform t es may be pre ented or l m ed by attent on from the first day of llness to phys o log c pos t ons of ad antage mod fied temporar ly to rel e e pa n or muscle spasm Turn ng *the pat ent n accordance w th need and assur ng* adequate freedom for breath ng w ll add to comfort by help ng c rculat on l m t ng pressure on sens t ve affec ed muscles and p o ecting aga nst pass e congestion in dependent parts

Mod fied spl nts and supports *espec ally useful* n pat ents w th resp ratory paralys s ha e been *seen in the demonstrat ons at th s conference* Careful pos t on ng of body parts nclud ng the thorax and sp ne at appropr ate inter als s n effect spl nting and may be successful in cooperat e pat ents but not usually n young ch l

dren many of whom require more formal support of extremities or trunk

In the event of asymmetric paralysis resulting in expansion of one side of the chest more than the other, a tendency to scoliosis will result A flat sandbag shaped to the contour of the better half of the chest may be made and anchored so as not to interfere with expansion of the limited side The weight and size of the sandbag on the good side should be planned according to the degree of inhalation possible on the more involved side so as to avoid too much restriction of total air intake

Movement of the joints of the spine thorax and extremities increases kinesthetic sense and makes the patient more aware of the area to which effort is directed Improvement of circulation gives better distribution of oxygen to all body parts Such valuable aid is given by the rocking bed besides its aid to breathing Symptomatic relief is then more easily explained because stasis of blood flow is not likely

Tolerance for physical treatment or appliances may be influenced by respiratory function as well as by electrolyte balance nutrition and cardiovascular functional capacity Transitions from respiratory aids to regular bed to sitting standing and walking are naturally slower in a patient with limited capacity for breathing If one does not appreciate this fact and proceeds to permit fast changes in the program the patient may regress so that return to mechanical help for this vital function results

Extremity bracing will follow the same indications as in patients without limited breathing except that lighter weight materials are used to limit exertion But modification of trunk supports will follow lines already mentioned as giving particular attention to holding the lower abdomen while not restricting diaphragmatic or costal excursion

Each patient with respiratory paralysis offers a special challenge for formulating a long range plan which will be influenced not only by problems in breathing but also by emotional and personality characteristics by extent of involvement of other muscles of the neck trunk or extremities as well as by the age size and physical endowment of the individual With the Salk vaccine we can look forward to diminishing numbers of new cases of poliomyelitis More professional personnel should be available for patients with respiratory paralysis so that we can do more efficiently what we know is needed to make the best recovery possible in the shortest time

DISCUSSION

Dr Pollock In expressing my gratitude for the opportunity which has been given to me to discuss Dr Wright's paper I must at the same time record the pleasure I have had in reading her excellent communication

The increasing degree to which specialization has overtaken our profession has tended to focus medical attention on progressively narrowing fields of interest so that to many of us the part is now almost more important than the whole It is both refreshing and invigorating to know that to Dr Wright the patient is indivisible and from the viewpoint of treatment must be considered as a total individual

She states rightly that the first step in treatment is to establish early in the disease a true doctor/patient relationship since only by so doing can we obtain his active co-operation and if we hope to retain it we must be as honest in our approach as we are realistic in our aims

In discussing physical medicine and orthopedics in the treatment of patients with respiratory paralysis I need make no apology for referring in particular to the most serious sequel with which the orthopedic surgeon has to deal in the convalescent stage of poliomyelitis—scoliosis There can be few deformities more crippling to body and soul than the progressive lateral curvature of the spine which may follow a widespread poliomyelitis

Every case of severe paralytic poliomyelitis is a potential scoliotic yet many cases show little or no evidence of this complication Others again with lesions apparently comparable in degree and extent develop a grossly deforming curvature Can any one of us with confidence name the essential cause? Ghormley suggested with more or less general acceptance that fascial contracture muscle imbalance and stretch paralysis were the exciting factors in its development But this is an oversimplification of the complicated interplay of the many factors which give rise to scoliosis and gives little or no guide in the detection of the structure initially at fault and in consequence an indication for its arrest or correction

In a recent review of 330 cases of poliomyelitis admitted to the Princess Margaret Rose Hospital Edinburgh during the 10 year period 1947 1957 51 cases showed evidences of respiratory involvement—many of whom required up to 3 months in a respirator

Of these 51 cases no less than 34 developed a scoliosis—a much higher incidence than that for the group as a whole It is not without significance that in this group alone i e that showing respiratory involvement a scoliosis was discovered clinically in 12 cases before the child was allowed out of bed and therefore before gravity could play a role in its production

It seems reasonable to assume that weakness of the long spinal muscles the sacrospinalis the quadratus lumborum trapezius and iliopsoas etc of one side—like the slackening of the guy ropes of a tent—will result in a spinal tilt to the opposite side or the reverse will occur if there is contraction In one case progress may go on to deformity and in another the condition remains unchanged

Examination of 21 cases by fluoroscope suggests that the weight which may tip the balance in favor of a progressive scoliosis is paralysis of one leaf of the diaphragm with a segmental or more widespread weakness of the intercostals of the same side occasionally aggravated further by involvement of neck and lateral abdominal muscles and at a later period by the effects of gravity and leg inequality in length and muscle power

In many of these cases the application early in the postfebrile period of the principles of treatment suggested by Dr Wright may limit if it does not completely arrest the tendency to increasing deformity

We know from experience that the acme of functional recovery following poliomyelitis can be obtained only where in addition to recovery of muscle power there is present a normal range of joint movement It is in the light of this fact that Dr Wright's insistence upon maintaining the costovertebral joints mobile must be viewed Also Cobb has shown that it is as important to mobilize the joints of the spine as it is to keep the articulations of the extremities supple



Fig 342 Gross lateral scoliosis with erosion of the ribs In this case the convexity was so gross that erosion of the ribs has taken place as a result of pressure



Fig 343 S oliosis showing spinal deformity



Fig 344 Deformity of rib which accompanies a severe scoliosis Note the extreme degree of obliquity of the ribs

Therefore it is only logical that we should apply to the costovertebral and fa et joints those methods of treatment advocated by Dr Wright and already in common use where the joints of the limbs are affected

Figures 342 and 343 show the extreme degree of spinal deformity The first one looks like a kyphoscoliosis but is in fact a gross lateral scoliosis with almost erosion of the ribs Roaf has recently pointed out that the axis of vertebral rotation in the dorsal spine corresponds to a point just anterior to the posterior margin of the body We know that this corresponds to the nucleus pulposis and that under normal conditions a line drawn through the nucleus will lie just anterior to the heads of the ribs Normally these exercise a balanced pressure on the body and transverse processes Roaf claims that in a scoliosis the position of the rib relative to the axis of rotation of the vertebral body changes so that the ribs on the convex side tend to fall behind the axis of rotation whereas those on the concave side lie in front of it Figure 344 seems to suggest that this is so but in addition it does demonstrate the degree of deformity of the rib which accompanies such a severe scoliosis—a point of considerable importance for not only do the ribs lie in extreme obliquity but they become hooked at the neck in such a way that any pressure applied to the convexity of the rib in an attempt to lessen the rib hump will cause direct pressure upon the transverse process and thus may lead to an increase in rotational deformity rather than to its diminution or correction I think that in the figures particularly on the convexity you can see the hooking of the remnants of the ribs and how they may press

on the facets Therefore in the *process of* mobilization of the costovertebral joints it is essential that pressure be applied correctly if Dr Wright s *objectives* are to be achieved Perhaps the fiercest spur to intensive and early *physical* therapy is the late results of surgery An operation to fuse the spine all too frequently is the final dramatic act in an attempt to arrest a progressive deformity which has deteriorated in spite of every other form of therapy Unfortunately spinal fusion is not as successful a solution to our difficulties as we would like to believe *In* a recent review of over 600 cases of scoliosis in which 58 cases (or 7 /) were successfully subjected to a spinal fusion operation *Winchester found that only 14 per cent showed* no further relapse That failure to fuse the spine over a sufficiently extensive area may have been responsible for some of the relapses is possible but the greater number were due almost certainly to two factors frequently overlooked (a) in attempting to produce a spinal fusion we are apt to forget that we are dealing with a growing structure subject to Wolff s laws of stress and strain and (b) that the initial causative factors are still acting Therefore it is not altogether surprising that so long as the original etiologic factors continue to exercise their deforming influence the growing spine will still respond and some degree of relapse is consequently almost inevitable A more critical physiatric analysis of the essential etiologic factors may lead to the future use of myotomy or neurectomy of the contracted or overacting muscles with arrest of the deforming process and a better end result than that obtained at present by surgery

At the moment our sheet anchor in the prevention and treatment of those deformities which follow paralytic poliomyelitis with respiratory involvement is the early and wise use of those physiotherapeutic methods so admirably described by Dr Wright

By the adoption of Dr Wright s method of treatment fascial contractures are stretched muscle tone is increased and pulmonary function improved

Increased vital capacity with improved oxygenation of the blood and better cardiac function must react favorably on muscular function generally and so lessen the tendency to spinal curvature and rotation and thus establish a salutory cycle of improvement in place of that *vicious one whi h leads* on to deformity

Dr Rae Dr Wright has emphasized that all professional personnel concerned with the treatment of respirator patients should be oriented to the multiple needs of these patients during convalescence The full co-operation of *medical* and ancillary personnel is required to bring these patients to maximal function and independence commensurate with their limitations The ultimate goal is to return the patient to as normal an environment as possible In many cases severely *involved* patients have been able to *return to productive employment* Social service workers and vocational counselors contribute significantly to the restoration program

I agree with Doctor Wright that physical treatment should not be the entire theme of the patient s program Appropriate counselling guidance and planning in psychological social and vocational areas should proceed at an appropriate pace There needs to be a correlation between such planning and the physical treatment program

There is little doubt that physical treatment plays an important role in the treatment of the after-effects of poliomyelitis and the functional restoration of the patient A program of physical care designed to promote a maximum of functional restoration must include measures to prevent and minimize deformities and mobilize the patient to as high a level of efficiency in body mechanics as possible to co-ordinate and strengthen neuromuscular units in which function remains and to make effective use of assistive devices orthopedic apparatus and surgical procedures

Although Dr Wright was unable in the time allotted to give a detailed description of the physical treatment of poliomyelitis she presented some interesting ideas regarding mobilization of the joints of the spine and the thorax She has pointed out that these joints are often overlooked in the mobilization program and that freedom of motion in these joints is necessary for optimum breathing One technic that seems to be effective in mobilization of the chest and that was not mentioned in this paper is glossopharyngeal breathing Glossopharyngeal

breathing is a technic for ventilating the lungs that does not require the use of the muscles of respiration The muscles of the mouth and pharynx are utilized to force air into the lungs under positive pressure This type of breathing enables patients with reduced vital capacity to inhale a far greater amount of air than is possible with any of the deep-breathing machines Some studies we have carried out seem to indicate that a more compliant chest can be obtained by periodically expanding the thorax and lungs with deep glossopharyngeal breaths The work of breathing is reduced with a more elastic chest In some patients this may mean more time free from respiratory aids or in some cases it may permit the use of a less confining respiratory aid

One of the most distressing features of poliomyelitis is the tendency to develop deformity for the disease is one of tissue contracture as well as of muscle weakness If spasm which occurs so frequently in the acute phase of the disease is allowed to persist permanent shortening of muscular and ligamentous structures may result Appropriate physical measures can minimize or prevent such tissue contractures The primary cause of deformity is persistent faulty position which may be the result of untreated muscle spasm muscle imbalance forces of gravity or poor body mechanics

In the acute phase of poliomyelitis adequate rest of course is essential but this does not mean that the patient should be immobilized completely Physical treatment should be started as early as possible and may consist merely of the use of heat often in the form of full prone packs and gentle passive motion to keep the patient mobilized

It is not always desirable to obtain a completely normal range of motion in all joints but merely to provide adequate mobility for functional purposes Many of the respirator patients with severe involvement of all four extremities and trunk will never be able to walk again and therefore need only sufficient range of motion to permit them to sit comfortably and to be dressed easily In fact in some patients it may be advantageous to leave some tightness to support severely weakened muscles Mild to moderate symmetrical tightness of the back might be used to stabilize the trunk when sitting Mild residual shortening of the muscles about a flail shoulder might minimize separation of the glenohumeral joint that may occur when the patient is upright and the arms are in a dependent position Normal alignment of body segments usually can be preserved by maintaining or restoring muscle and joint mobility by proper positioning of the body and by using apparatus to support weakness

Various types of orthotic devices and braces may be of great value in (1) prevention of deformity (2) support of weakness (3) assistance in the performance of useful activities Orthopedic devices and braces should not be employed as merely a last resort but used intelligently to achieve any of the above objectives

I would thank Dr Wright for an interesting paper and for bringing to our attention certain concepts and new technics for mobilization of thoracic structures

Treatment of Early Paralytic Scoliosis

DR ROBERT BENNETT

Early recognition and treatment of paralytic scoliosis is necessary to prevent the development of significant structural distortion of the spine thorax and pelvis Not all of the factors that cause persistent lateral and rotary deviations of the spine can be prevented or corrected but the development of serious structural changes can be controlled in most cases by adequate care Preventing correcting or minimizing scoliosis depends on (1) *very early recognition of persistent* lateral and rotary deviations of the spine (2) recognition of all factors that could possibly bring about persistent faulty alignment of the spine (3) *constant attention by the physician* (4) full co-operation of the patient and his family

X ray visualization of the spine is necessary *to detect the earliest deviations from normal* alignment Such roentgenograms should be taken in the erect sitting or standing position Roentgenograms taken in the supine position are *of little value because they minimize the effect of* weight and the attempts to stabilize the spine by weakened musculature or poorly balanced muscle strength Because roentgenograms should be *taken early in the course of paralytic disease* sitting roentgenograms are preferable to standing views Patients with severe paralytic disease can be brought to a sitting position safely long before they can be permitted to stand When spinal deviations exist particularly in the growing child roentgenograms should be repeated every 3 or 4 months to determine the response to treatment and the effect of increasing age weight and activity It is always wise to x ray the spine both with and without supportive and corrective apparatus to be sure that such apparatus is fulfilling the purpose for which it was applied

By clinical and x ray visualization early paralytic scoliosis may be expected to follow one of four patterns or types Occasionally in the very early stages several types may occur simultaneously Combined types are common and expected in the later stages of the development of severe structural scolioses An understanding of the basic types makes evaluation and treatment of the combined types less difficult and far more specific An understanding of the pathogenesis and significance of the individual types enables us to analyze the various combinations that may occur

Type 1 This type is characterized by (1) a lateral tilting or shifting of lumbar 4 on lumbar 5 on sacral 1 (2) a compensatory curve in the mid or high lumbar region (3) a level pelvis *when patient is sitting or standing There are* five important causes of type 1 These causes may act singly or in multiple combinations (1) pre-existing developmental faults in the lumbosacral region (2) weakness or imbalance of intrinsic spinal muscles (3) weakness or imbalance of quadratus lumborum and/or iliopsoas (4) asymmetrical contracture of the iliotibial band which may occur on either side of the convexity of the curve (5) asymmetrical loss of bulk at the hip and thigh This type is significant because faulty lateral take-off at the base of the spine puts a strain on the entire spinal column and tends to exaggerate any weakness and imbalance that might influence the alignment of the spine above the lumbosacral region The faulty lateral take-off may accelerate a curve existing above or conversely may act as an anchor to control a curve above Further deviation in the lumbosacral region with increase in the lumbar curve may be expected (Fig 345)

If the faulty lateral take-off in the lumbosacral region cannot be corrected one of three patterns of progression may be expected

1 The ilium on the side of the acute lumbosacral angle may be raised and the spine above regain normal alignment

2 The lateral take-off will increase and the lumbar curve secondary to this deviation will progress rapidly The dorsal spine will remain in good alignment except for its lower portion



Fig 345 Type 1 scoliosis Early (*left*) and common pattern of progression (*right*)

which has become the base of the lumbar curve The rib cage will not be significantly distorted

3 A compensatory curve will develop in the dorsal region to balance the lumbar curve This dorsal curve may progress out of control cause severe distortion of the thorax and become the major problem In this pattern the lumbar curve usually decreases as the dorsal curve progresses

Treatment of a type 1 pattern should emphasize the following 6 points

1 Every attempt should be made to mobilize immediately the acute lumbosacral angle This may be done by passive or active stretching X ray visualization of the lumbosacral region must be available not only to determine the extent of deviation and its response to treatment but also to determine if a structural fault exists that would limit the possibility of realignment of the spine with treatment (Fig 346)

2 If asymmetrical contracture of the iliotibial fascia exists it must be released immediately either by conservative stretching or fasciotomy Conservative stretching obviously should be attempted first but if there is not a very definite release of contracture in a reasonably short period of time surgery must be carried out In the early stages of the development of a type 1 asymmetrical tightness of the iliotibial fascia is equally important on either side If it exists on the side of the deviation it will pull the pelvis down increasing the lumbar curve If it is



Fig 346 One method of stretching the left lumbosacral angle without danger to the left lumbar curve

present on the side opposite the lateral deviation it may stretch out the lumbosacral region and thus increase the lateral deviation

3 A gluteal pad under the side of the acute lumbosacral angle should never be used without very serious consideration and x ray study It is always a temptation to realign the lumbar curve secondary to the lumbosacral deviation by lifting the pelvis on the side of the convexity of this lumbar curve Unfortunately this usually results in an increase of the lumbosacral angle

4 As stated previously one of the greatest dangers of this type of early deviation is the later development of a compensatory shift of the thorax to the side opposite the lumbosacral deviation This shift of the thorax takes place rapidly in growing children particularly after 9 years of age In a pure type 1 deviation uncomplicated by severe trunk weakness this is by far its most important aspect and must be counteracted by support and stretching

5 A true type 1 pattern of deviation cannot be controlled or significantly altered by any type of back brace or corset There is no way through spinal support to obtain pressure low enough in the lumbar region to affect the lumbosacral angle Spinal traction is of some limited value and should be attempted This can be accomplished by repeated hanging from an overhead bar use of Sayre head sling attachment of a head traction to a corset or back brace However traction may prevent the later shift of the thorax and certainly should be used when there is any indication that this shift is taking place A firm well fitting corset with its top shifted off against the shifting of the thorax should be applied immediately if such thoracic shift is noted





FIG 347 (*Left*) Type 2 scoliosis (static) (*left*) with gluteal pad under right hip and thigh (*right*)

FIG 348 (*Right*) Type 2 scoliosis (dynamic) Early (*left*) and common pattern of progression (*right*)



FIG 349 Type 3 scoliosis Early (*left*) and common pattern of progression (*right*)

6 Any activity that causes downward pressure against the lumbosacral region should be sharply limited This means that there should be marked restriction of both sitting and standing periods The patient should be kept in a reclining or semisitting position as much as practical and consistent with the care of other skeletal problems

Type 2 This pattern of early spinal deviation is characterized by (1) a pelvic tilt downward on the side of a midlumbar convexity and (2) a normal lumbosacral take-off This type must b recognized as having two forms the static and the dynamic The static form is common It is simple to correct and important only as it influences other types of deviations The dynamic form is fortunately not common It is almost impossible to correct It usually results in severe structural changes in the spine and in over*all* functional handicap

We will discuss the static type first Figure 347 shows stretching of the lumbosacral region and was a part of the discussion of type 1 This is simply one method for stretching the lumbosacral region This is a typical static type 2 the common causes of which are

1 Asymmetrical loss of bulk in the hip and thigh causing the pelvis to tilt when the patient is sitting

2 Discrepancy in lower extremity length tilting the pelvis when the patient is standing

3 Faulty fitting of lower extremity apparatus so that the patient tends to sit down in his brace The brace may be too short above the knee or the upper thigh band may be too deep or the knee pad too loose Tilting of the pelvis for these reasons causes a simple functional curve that has little importance in itself However it may act as an accelerating factor in the progression of other curves

I think the treatment of type 2 is obvious so we will go on to the dynamic which is far more important

Dynamic Type 2 This form is caused by severe imbalance of strength of trunk musculature of hemiplegic pattern of imbalance This





Fig 350 (*Left*) Straight corset (*left*) and with top shifted to left (*right*)
Fig 351 (*Right*) Roentgenogram of patient with and without shifted corset

imbalance is almost invariably coupled with iliotibial fascial contracture on the side of the weakened musculature. The pelvis is kept in a persistently tilted position by the unopposed use of lateral trunk musculature lifting the patient on the side of the strength and the tight iliotibial fascia depressing the pelvis on the opposite side. Figure 348 shows the progression of such a curve. The treatment of this dynamic pattern is extremely difficult.

Certainly the iliotibial fascia on the low side if tight or snug should be stretched or its mobility periodically determined and then sectioned by surgery if it cannot be otherwise mobilized. In this type we believe that a corset with head traction should be utilized for its pressure against the pelvis in the attempt to help the imbalance.

Type 3. This is a common type characterized by a long C curve. It may occur in any level. Figure 349 shows a simple one of the dorsal lumbar region and its usual form of progression. It shows a curve slightly higher still of type 3 and on the right its common progression. It is characterized by having a normal take-off in the lumbosacral region and a fairly level pelvis commonly combined with a type 1 deviation and if such a combination exists a severe single or even a double curve may be present very early.



Fig 352 Type 4 scoliosis. Early (*left*) and common pattern of progression (*right*)

The treatment is to obtain and maintain symmetrical mobility of the spine and is the key to the control of this deviation. This does not mean that normal mobility is desirable in each case. If muscle weakness or imbalance exists symmetrical tightness may be necessary to maintain alignment. In general it can be stated that mobility must bear a direct relationship to underlying strength.

Many different types of spinal support have been designed for this type of curve each has advantages as well as disadvantages. We use a basic Hoke type corset for spinal support as we feel that it can be made to fit well and can be

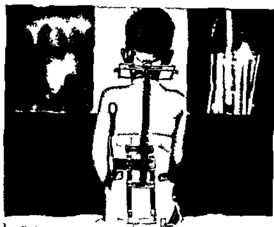




FIG 353 (*Left*) Adjustable head traction attached to corset (back view)
FIG 354 (*Right*) Adjustable head traction attached to corset (side view)

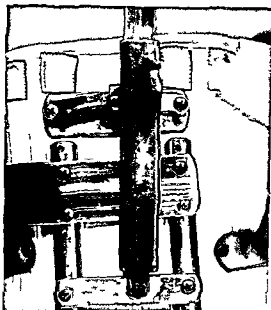


FIG 355 Traction bar may be adjusted both vertically and horizontally

reinforced Also it can be shifted at the top to the right or left to combat the tendency of a right or left dorsal lumbar curve of this type Figure 350 shows a simple corset with its top shifted

Figure 351 shows roentgenograms of a patient with a left dorsal lumbar curve and a corset with its top shifted to the right showing the influence of the underlying spine

Type 4 This pattern is characterized by an angular curve at the level of dorsal 4 5 or 6 and the existence of a long moderate dorso-lumbar curve with minimal rotation Usually there is a good take-off in the lumbosacral region and the pelvis is fairly level The causes of this type of curve are not completely understood Certainly weakness or imbalance of the intrinsic spinal musculature of the upper dorsal region must be at fault In many of these patients we find rather severe loss of strength in the muscles of inspiration and very frequently we find imbalance about the shoulder girdle This curve is of great significance because it tends to develop the severe structural curve of great cosmetic as well as physiologic importance Figure 357 shows the typical pattern of progression

As previously noted early recognition is the most important point in treatment of type 4 Certainly conservative methods of care are incapable of correcting significant structural scoliosis Prevention of significant structural scoliosis is possible but can be achieved only through early recognition and control of all those factors present in the individual patient that might cause persistent faulty alignment of the spine General treatment will consist of (1) limited activity (2) corrective mobilization and muscle strengthening (3) spinal support and (4) control of bodily weight (Figs 353 354 and 355)

Treatment is tedious and requires the constant attention of the physician and the full co-operation of the patient and his family A knowledge of the pathogenesis of the four common types of early deviation of the spine is of great help

in the prescription of care for these individual types as well as in determining the important features and the care of combined types

BIBLIOGRAPHY

Bennett P L Classification and treatment of early lateral deviations of the spine following acute anterior poliomyelitis Arch Phys Med 36 9 1955

—— Head traction attached to corset in care of scoliosis Arch Phys Med 32 84 1951

—— Orthetis for function—Part I Phys Therapy Rev 36 771 1956

—— Supportive and assistive apparatus in *convalescent poliomyelitis in Proc Internat* Congr Phys Med 1957

Knowlton G C and Bennett R L Overwork Arch Phys Med 38 18 1957

of our advice to the contrary to cope with the child s natural tendencies toward activities and widely unrestricted movements

Duly appreciating the endeavors of my colleagues and particularly of Dr Bennett to look for conservative measures in preventing severe paralytic scoliosis and trying myself to do the utmost in the same direction I must confess that at least with little children and under difficult social and economic conditions the outlook for scoliosis originating from paresis or paralysis of intrinsic structures of the back and treated by conservative methods is not favorable

REFERENCES

1 McCauley J C Poliomyelitis Papers and Discussions Presented at the First International Polio Conference p 226 Philadelphia Lippincott 1948

2 Knapp M E Treatment of acute poliomyelitis at the Kenny Institute *in* Proc First Internat Congr Phys Med p 110 London 1952

3 Grossiord A and Ciosi Frenay C A propos de la prevention des scolioses poliomyelitiques European Association Against Polio Third Symposium Zurich Sept 29 30 1953

4 Cobb J R Correction of scoliosis *in* Poliomyelitis Papers and Discussions Presented at the Second International Polio Conference p 250 Philadelphia Lippincott 1952

5 Bennett R L Physical medicine in poliomyelitis—points of emphasis *in* Poliomyelitis Papers and Discussions Presented at the Second International Polio Conference pp 263 264 Philadelphia Lippincott 1952

6 Grossiord A Bourdon P Ciosi Frenay C Held J P and Beaupere G Etude critique de l examen radiographique du rachis poliomyelitique Sem Hop Paris 32 1484 1956

7 Grossiord A Ciosi Frenay C Held J P and Beaupere G Les paralysies abdominales dans la poliomyelite leur bilan clinique—leur role dans la genese des scolioses *in* Proc Second Internat Congr Phys Med p 499 Copenhagen 1956

8 Webster F S The problem of scoliosis—its evaluation treatment and prognosis Nebraska M J 35 115 1951

9 Moe J H The management of paralytic scoliosis South M J 50 67 1957

10 Barr J S The management of poliomyelitis the late stage *in* Poliomyelitis Papers and Discussions Presented at the First International Polio Conference p 210 Philadelphia Lippincott 1948

11 Grossiord A Tournilhac M and Verley R Prevention des deformations dans la poliomyelite Hospital Paris 44 56 1956

12 Grossiord A Ciosi Frenay C Held J P and Beaupere G A propos de la prevention des scolioses poliomyelitiques *in* Proc Second Internat Congr Phys Med p 491 Copenhagen 1956

DR GROSSIORD I congratulate the speaker on his very interesting report nearly every line of which is worth meditating On the whole I am in full accord with the remarks made on the four principal types described by Dr Bennett

Our topic is a highly specialized one as is that of the paper and the exhibit my coworkers and I have prepared on the subject the consideration of the possibilities for prophylactic treatment of poliomyelitic scoliosis that is to say resolute consideration of the purely medical aspects of the problem when confronted with a case of poliomyelitis of recent onset

Our experience is now based upon 9 years of work in our Centre de Reeducation de Garches (Garches Rehabilitation Center) and more than 2 000 cases of paralytic poliomyelitis most of them severe all having been hospitalized at some time and many of whom presented spinal complications For the past several years all cases of poliomyelitic spine have been subjected to minute investigation and given standardized clinical x ray examinations

If we attempt to summarize in a few words the pathogenesis of poliomyelitic scoliosis which is in reality extremely complicated we find four essential factors

1 *The muscular imbalances created by the* disease which produces this condition to perfection and it seems to us beyond question that the involvement of the spinal muscles themselves is by far the most important in this connection the danger arising from other possible factors of imbalance seems to be limited Actually we must first agree on what is to be called normal spinal musculature[?] Slight involvements discoverable only by delicate diagnostic procedures must not be overlooked

2 Aponeurotic fibromuscular contractures and here again directly paravertebral contractures are of greatest importance

3 Aggravating effect of "mise en charge

4 Disorders no less important of growth according to the old Law of Delpech

Granting that although everything will be done to perfect attention and nursing in th acute stage and to see the patient through the first or second month in the best possible condi tion we are nevertheless bound to admit a number of points

1 A muscular imbalance if it is definite never will be completely compensated the antagonistic muscle pair regaining strength but remaining imbalanced

2 When a well-defined s ate of contracture exists in the paravertebral structures it is diffi cult to reduce and if that can be achieved to maintain the results obtained "postures dorsal or decubital frames corsets etc have only limited effect

3 Apparently we can do nothing against the growth itself of the spine at least by simple measures

4 Thus there remains to us in the last re sort one factor above all upon which we can act when there is danger the management about mise en charge we can in fact control it to some extent.

SELECTION OF CASES

The danger of scoliosis in paralytic poliomye litis is such that it is essential to be able to say with rela i e precision in a given case whether or not the spinal column is in danger

This evaluation scarcely can be made in the first weeks

In general it becomes possible only at the end of the first month sometimes a little later and we believe that strict decubitus should be maintained so long as ne has not been able to form an opinion What this actually means is that the sitting position is ruled ou during the first month of the disease This spinal balance sheet will be

1 Clinical ba ed on detailed testing of the trunk muscles above all those of the spine

2 Radiologic based on three standard frontal plates

(a) corrected de ubitus

(b and c) forced lateral infl xions right and th n left in the same position

We may certainly agree with the speaker that a plate of the lying position minimizes the danger which a plate of the sitting position objectifies but it may be objected to this that a plate of the sitting position is subject to many

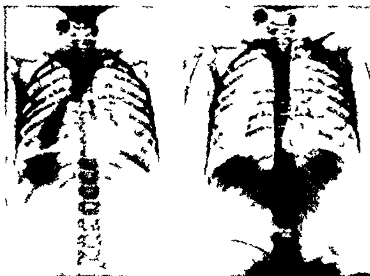


Fig 356 Aspects of rotation redu ed by use of a pillow under the atrophic buttock

causes of error certain of which are detailed in our exhibit and that these causes of error are such that the radiologic evidence will lack the comparative value needed in order to observe the development properly A corrected plate of the lying position presupposes correction of pelvic imbalances from atrophy of one buttock (Fig 356) lowering of one half of the pelvis by retraction of the tensor (Fig 357) if necessary a cushion is placed under the atrophied buttock or one thigh is flexed while verifying the compensation on the iliac spines by using a spirit level The pelvis being perfectly level any rotation (Fig 359) or inflexion becomes significant and can b cal ulated

To this plate of corrected decubitus for the first radiologic balance sheet we systematically add two more decubital roentgenograms these in forced lateral inflexion the procedure being supervised by the physician Any noteworthy segmental asymmetry in extensibility should b considered as a serious sign of incipient scoliosi (Fig 358)

Thus we believe that we are able to differentiate with this clinical x ray balance she t

1 Cases in which the danger appears to be absent or virtually absent the trunk musculature has been spared and the spine is completely normal the spinal column is well aligned and its lateral inflexions quite symmetrical the prognosis is very favorable in all likelihood no scoliosis will appear later even if the child has one leg shorter than the other We will not say that the possibility of scoliosis appearing is nonexistent caution always is indicated and the spinal column always should be kept under close observation after poliomyelitis We shall say only—but this is on the basis of considerable follow up—that whenever we have based such a prognosis upon wholly favorable elements the facts have never failed to bear us out Perhaps they may so fail tomorrow but we do not believe they will

2 Cases in whi h there is danger the gravest doubts must be entertained and no attempt should be made to rush through the stages of re-education We remarked above that limiting the mise en charge is one of the surest means of reducing the danger

The various aspects of the treatment certainly should be accompanied by all necessary rehabilitative measures hot water balneotherapy analytic exercises to strengthen weak muscles measures to combat contractures closely fitting plaster casts at night with corrective hyperextension when possible

Re-education in standing and walking posi

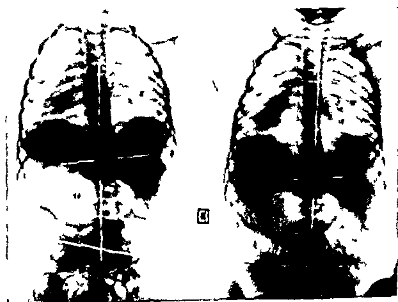


Fig 357 Scoliosis largely reduced by the correction of the pelvic imbalance (due to the retraction of the tensor of the fascia lata)



Fig 358 The aspect n corrected prone de cub tus is not normal The nflex ons a e asym metr cal The pat ent was lost to fol o v up Subsequent develop ment of marked scol os s



tion wh ch is infin tely less harmful than the s t ting posture undoubtedly should be begun q te early

Certa nly e eryth ng should be done to ensure that the ch ld s essent al phys olog c needs are met in sp te of the treatment

But v e are firmly conv nced that in th s effort to reduce the danger of scol os s when one knows t to be present in a case of pol omyel t c sp ne postural restr ct ons as to perm ss on for the stand ng posture and abo e all the s tt ng pos t on play an important part for 6 12 or 18 months and somet mes longer

An infin te number of types of cases is po s ble in pract ce

(a) the most favorable in wh ch the sp nal muscles beg n to reco er the r s rength very qu ckly and the sp nal column rema ns per fectly al gned—cases in wh ch great l berty of mo ement w ll be allo ed qu ckly wh le keep ing the sp nal column under very close observa t on by means of corrected decub tal plates e ery 3 to 5 months

(b) the most se ere wh ch v ll be kept lying down most of the day for a long t me except for per ods of ambulatory re-educat on

(c) those cases in wh ch one w ll perm t 2 hours stand ng posture da ly for example but no s tt ng or complete freedom to stand but not s tt ng or f eedom to stand du ng the morn ng and to s t at meals et

The means for carry ng out the e essent al measures is afforded by the char ot plat n all ts poss ble var ants ncl ned or not h ch the ch ld takes v th h m on va at ons th h s fam ly h h he uses in go ng to school and on vh ch he plays

It s certa nly not s mple to ga n acceptance for t the parents must be con nced wh h is not at all mposs ble and exper ence shows that the results pay off Not that we pretend to el m nate the danger of scol os s by these restr c t ons for gro th cont nues e en when the child s ly ng do vn but v e bel e e that t would be infin ely less harmful to the sp ne of a ch ld ly ng down 20 or 22 hours out of 24 than n a

child permitted to sit even though he be well supported by a corset and *a fortiori* if he is not —which you will agree is common enough

However in practice we encounter a major difficulty connected with age

If it is a question of a very young child 1 or 2 years old whose condition is such as to portend the later development of scoliosis purely medical means would be of little avail one could limit the risks to be sure but with 10 12 or 14 years of growth ahead how can one insure proper precautions? The solution is to be found only in repeated orthopedic correction by means of special plaster casts or in surgical

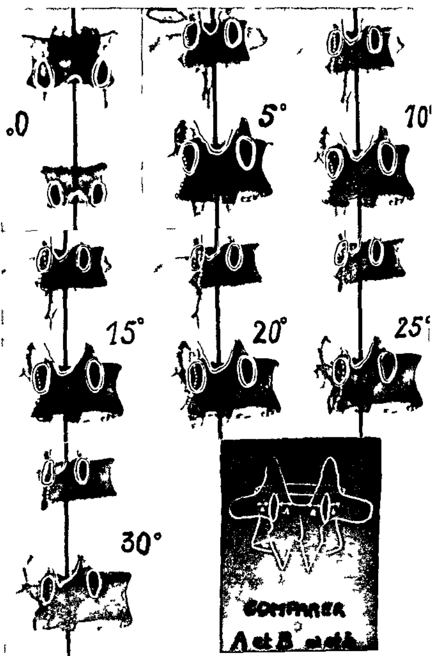


FIG 359 Table of rotations

measures But what is one to do in the case of the very young?

However if we are faced with a child of 11 or 12 for whom the dangerous years of growth are now limited we believe that the danger is great enough so that we can strongly recommend the restrictions of the application of *mise en charge* of which we have spoken These will be more or less long lasting and more or less stringent but we have come to admit the fact that in certain difficult cases in which nevertheless victory appears probable these measures are of value for several years We have followed and are still following numerous patients who have undergone such measures Let no one object with generalized psychological arguments stressing how painful it is thus to keep a child flat on his back for 2 or 3 years! First of all decubitus often is not strict at all many children walking it is the sitting position we fear the most In addition we have been able to show that with the use of proper precautions such restrictions are often much better accepted than one would expect and that the children suffer relatively little from them Finally the result of bringing these children to age 15 with a straight spine justifies all these efforts both from the physical and psychological point of view

Let us make it clear that we had no intention of addressing ourselves in this brief paper to the problem of treating cases in which scoliosis of some magnitude is already established this problem is one for full scale orthopedics What is of interest to us within the scope of our work is preventive treatment such as

1 Preventive treatment in which the constant care in the acute phase is unquestionably the best possible rapid alleviation of pain and spasm close supervision of posture

2 Preventive treatment in which selection of cases of spinal column in danger is a prerequisite the selection necessarily being based on faultless clinical x ray semeiology

3 Preventive treatment which in our view must in every suspect case be based essentially on a systematic and cautious approach with regard to the application of *mise en charge*

We shall give no figures as to the length of treatment for they depend on the circumstances of each individual case but it seems quite clear that such an approach permits

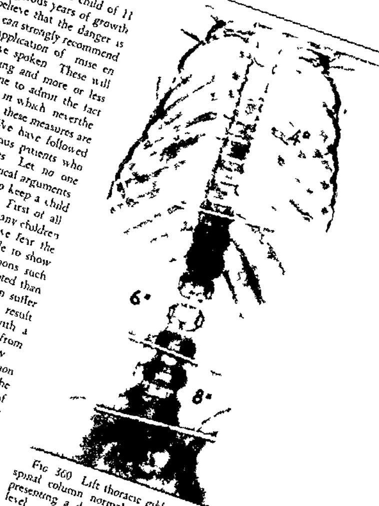


Fig 360 Left thoracic gibbus facing a spinal column normal at that level but presenting a discrete scoliosis at a lower level

(a) the closest supervision of the spinal equilibrium over a longer period of time

(b) strengthening of the trunk muscles and consequent reduction of the harmful effects of the first liberties of *mise en charge* and

(c) gaining ground on the danger period a gain which will to be sure be negligible or nearly so in the very young but considerable in those 12 or 13 years old

Actually in this paper we have considered nothing beyond ordinary cases our clinical material not extending to severe chronic respiratory cases however we have frequently encountered

the extremely delicate problem of possible relations between progressive costal gibbosity (in which the ribs tend toward the vertical) and a dorsal spine which sometimes seems definitely to turn following the movement but which may also retain its normal appearance (Fig 360) Must not failure of the muscles originating at the apex of one hemithorax—scalenus and primary intercostals—be held responsible in certain of these cases? At any rate it is a fact that treatment of these cases is particularly difficult and when there is a dorsal scoliosis evolving rapidly along with a gibbosity of this kind postural restrictions will not modify it

Surgical Methods of Improving Scoliosis in Poliomyelitis Patients

DR CARLOS OTTOLENGHI

This is a criti al analysis of the surgical methods employed for the improvement of poliomyelitic scoliosis It will not be a statistical paper as that already has been provided most competently by Cobb in the Second International Poliomyelitis Conference in 1951 in Copenhagen

In our files we have records of 1 634 cases of scoliosis of different etiologies Before we enter into the surgical treatment we have to make some general remarks as follows

1 In paralytic scoliosis there is a muscular disequilibrium which we do not find in idiopathic scoliosis

2 The earlier paralytic scoliosis appears after the attack the more severe is its evolution

3 Poliomyelitic scoliosis does not appear or is mild in subjects whose growth was complete before paralysis occurred

4 The growth factor is fundamental for the prognosis evolution and treatment of poliomyelitic scoliosis

5 It is very important to know the evolutionary prognosis of the curves This has been studied by Ponseti and Friedman and by James James points out that in cases of thoracic scoliosis commencing between the ages of 0 to 3 years at the age of 10 the curves are above 70 When scoliosis appears between the ages of 5 and 7 at 10 years of age 80 per cent of the cases have a curve of 70 and when scoliosis

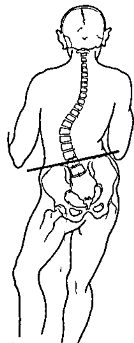


Fig 361 Upper oblique pelvis primitive scoliosis

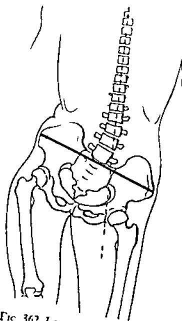


Fig 362 Lower oblique pelvis secondary scoliosis

appears at the age of 10 65 per cent at the end of the growth period have a curve of about 70 This means that we have a good basis to establish the prognosis

The objectives of surgical treatment are as follows

1 To correct progressive deformation
2 To avoid instability
3 To diminish fatigue and pain features generally present in older children or adult cases

Associated paralysis could be

1 Scoliosis without paralysis of the upp r or lower limbs
2 Scoliosis with paralysis of the upper or lower limbs

In our experience we have found that the paralysis of the scapular muscles does not affect or affects in a very small degree the appearance of scoliosis contrary to what was maintained by Colonna and Vom Saal

We have scoliosis with paralysis of the lower limbs which may be peripheral in which we find a shortening of the limbs and where very rarely do we find structural scoliosis with the angulation and rotation that characterizes true scoliosis Generally they are scoliotic attitudes Oblique pelvic scoliosis is of two types the upper and the lower

Figure 361 shows one type of oblique pelvis This would be the type of oblique pelvis with high variety primitive intrinsic scoliosis

Figure 362 shows the second type of oblique pelvis the low type s oliosis being secondary to deformation of the pelvis In these cases when scoliosis is secondary to deformation of the pelvis and when it is straightened scoliosis disappears (Fig 363) The curve is mobile and secondary to pelvic deformation These cases as Dr Bennett has pointed out must be studied following Irwin s principles

But the other type the high variety requires attention because if scoliosis is primary and rigid when the pelvis is corrected scoliosis becomes worse (Fig 364)

We shall now review other points that are of interest for the indication of surgery and they are as follows

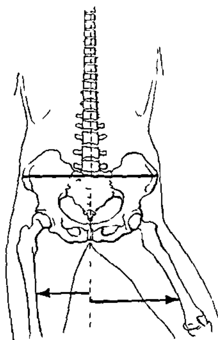


Fig 363 Correcting deformity in the pelvis makes the secondary scoliosis disappear

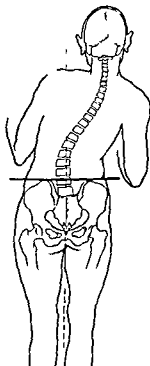


Fig 364 Scoliosis becomes worse when the pelvis is corrected if the scoliosis is primary and rigid

1 The longer the patient rests in a supine position during the growing period when suffering from paralysis of trunk and abdominal muscles the better will be the evolution of the scoliosis

2 In some cases in spite of being immobilized in bed deformation sets in This is possibly due to fascial contractures

3 Alternating gait is a serious factor tending toward a worsening of the scoliosis

4 Gravity in the absence of an efficient support worsens static conditions of the weakened spine

5 In patients who use crutches because of weakness of the lower limbs the curve has less tendency to increase

We must consider the over all aspect of scoliosis in these cases not only with regard to the spine in these cases particularly in the paraplegic cases we must try to see whether or not an improvement of the scoliotic curve also can condition an improvement of health in general This we must always take into account

We shall now review the different surgical methods

The diagnosis of scoliotic curves is easily done in cases of poliomyelitis because if we place the patient in the position shown in Figure 365 the primary curve is the one we find more easily Here you see some points that we consider of interest and importance

Correction of the curve must be done before the operation We understand that this is important and in this manner we can determine the number of vertebrae that must be corrected and the degree of flexibility of the spine Angulation and rotation is corrected by means of the Risser type corset following the different technics of this author The primary curve must be fixed if possible one or two vertebrae above or below the end

The operation can be done when correction prior to the operation exceeds 25 or 30 degrees if the curve in scoliosis is over 50 degrees If the secondary curve is not entirely corrected the primitive curve must be straightened or corrected only to that point where the secondary curve compensates it otherwise there will be an aggravation of the curve

Here is a routine case of a long curve 60 degrees (Fig 366) Once corrected we see it is straightened (Fig 367) Here it is in a plastic cast with a window and here are the marks (Fig 368) where we are to practice this operation This was the case of a young girl of 8

If the curves are flexible evidently in correcting the primitive curve we correct the secondary one but if on the other hand as we said these



Fig 365 Body positions for making diagnosis of scoliotic curves

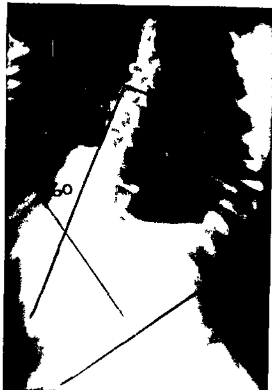




Fig 366 (*Left*) Routine case of a long curve of 60
Fig 367 (*Right*) Long curve of 60 after correction with plastic case (with window)

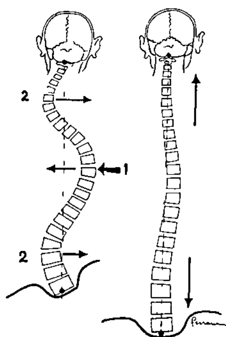


Fig 368 Diagram illustrating correction of primary and secondary curves

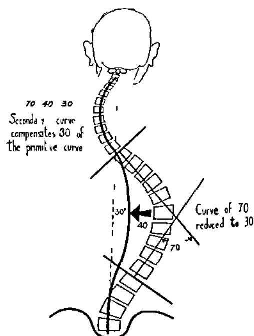


Fig 369 Diagram illustrating how the secondary curve is only corrected to 30 if the primary curve is corrected to 30°

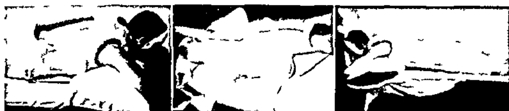


Fig 370 Patient in plaster cast showing how the plaster is open sideways and fastened with adhesive tape (3 positions)

curves are not flexible and if for instance this curve of 70 degrees is corrected only to 30 degrees the secondary curve also will have to be corrected to 30 degrees (Fig 369) to compensate or otherwise we would have a decompensation of the curve Naturally this is done on the basis of roentgenograms taken routinely

Some points concerning the technics are worthy of mention and they are the following

We have to be very careful in the preoperative period to make a respiratory and circulatory test Then during anesthesia and immobility we must be very careful We suggest that the operation should be done through a window but we open the plaster cast sideways because we have lost one patient 12 years old because we were not in time to open the plaster in order to practice thoracotomy

Figure 370 left shows a patient in bed and you see that we have the head up Here you see how the plaster is open sideways and how it is kept closed with adhesive tape because if anything goes wrong it is easy to open it Figure 370 middle and right illustrates the same thing

With regard to technic (Fig 371) during the growth period fusion must be done on the convex side when growth is ended on the concave side

In Figure 372 left and right we have the same examples as before showing that if the secondary curve is rigid it gets worse when we straighten the primary curve

We practice midway incisions through the window in the plaster cast We separate the tissue on one side only The muscular masses are separated on one side in order to operate on the convex side We go very far out we have to reach the articular facets and then following Hibbs technics we take advantage of the bone and we fill in the channel If there is room we

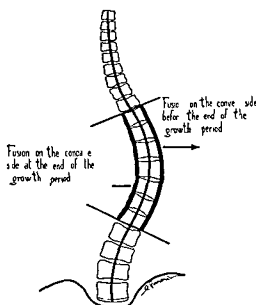


Fig 371 Fusion technic During growth period fusion must be done on the convex side when growth is ended on the concave side

put in a rigid graft We keep the patient in bed as long as necessary following a principle which says that immobility must be maintained as long as necessary

Treatment of paralytic scoliosis does not come to an end when fusion is solid and when the operation has finished because we must take into account that the bone is elastic subject to pressures and that the deformity forces may continue to act The difficulty that appears in these cases is pseudarthrosis the more frequent the more mobile the segment of the affected part of the spine and frequent in all statistics Both in our own statistics and those of other offices

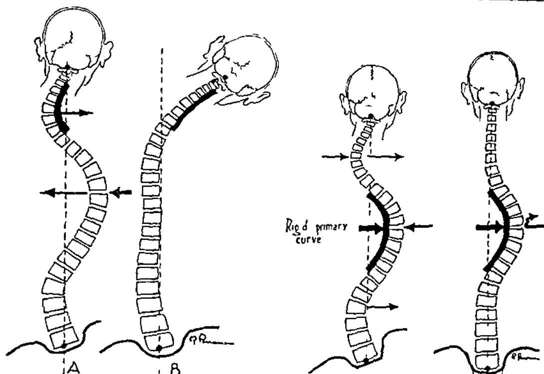


Fig 372 Illustrations showing how the secondary curve if rigid gets worse when the primary curve is corrected

we see that 6 months after the operation even in apparently solid fusion there is a loss of 25 to 30 per cent of the result obtained

We shall now consider other technics Vertebral osteotomies may be indicated in the fixity of the curve heavy primary curve and here we shall have the advantage of hearing Mr Roaf who no doubt will speak about it because he has developed a technic and has operated in 16 cases applying it

Figure 373 shows resection of vertebral bodies laminae and ribs in order to try to straighten a primary rigid curve If we take the primary curve and straighten the secondary we shall have a false reduction and scoliosis will continue (Fig 374) In these cases of accentuated scoliosis the osteotomy that Roaf speaks about would be the one that should be practiced We have very little experience with these cases and we think that this is still in the experimental stage We have not seen any especially good results Dr Harrington no doubt also will speak to us about osteotomies but we believe in the biomechanical technics and the rigid metallic devices placed in order to maintain the spine straight do not seem to us to be entirely satisfactory We are not sure of the points of pressure We think that with the lateral curve it can be used but where there is total deformation particularly through rotation we think that technically it must be difficult to place these devices Nevertheless we consider anything that is done to improve the condition of these patients to be welcome although in the first instance it would seem that the results perhaps would not be satisfactory

I shall now finish by saying a few words about the most important thing that is the age of the patients who are being treated As we understand it the most important thing is the growth factor therefore the younger the patient the more severe is the evolution of scoliosis and the more difficult its treatment The points under discussion are the following

1 Whether the fusion affects growth or not

2 Whether or not the fusion in the growing period can produce other curves as serious as those of scoliosis that is secondary scoliosis





Fig 3 3 (*Left*) Resection of vertebral bodies laminae and ribs in order to try to straighten a primary rigid curve

Fig 374 (*Right*) False reduction resulting from straightening the secondary curve

3 Whether or not operations at any age are truly effective to hold the progressive curves

We think that these are the most important points to be discussed We believe that in these cases with high progressive curves 100 per cent will have serious s oliosis at the end of growth after a period of observation as indicated by Dr Bennett and his collaborators If the curve increases we must have the courage as Risser says to reduce the scoliosis and operate on the patients at an early age In any case we are in the experimental stage in this field The results obtained are not entirely satisfactory but nevertheless just as we have avoided secondary deformation of poliomyelitis in the limbs we also believe that we should avoid the formation of scoliosis which affects the physical and moral aspects of patients In any case any operation that may enable improvement of the stabilization of the curves an improvement of the patient s moral health always will be helpful

BIBLIOGRAPHY

Allan F G Scoliosis operative correction of fixed curves J Bone & Joint Surg 37 B 9? 1955

Pertrand P Deux procedes operatoires nouveaux dans le traitement de scolioses graves Soc Intern Chir Orthop Taumat p 173 1954

Clarke O Modern Trends in Orthopaedics vol 1 p ?20

Cobb J R Outline for the Study of Scoliosis vol 5 p 261 Am Acad Orthop Surgeons 1948

——— Correction of scoliosis *in* Poliomyelitis Papers and Discussions Presented at the Second International Polio Congress p 250 Philadelphia Lippincott 1951

Colonna P and Vom Saal F A study of paralytic scoliosis based on 500 cases of poliomyelitis J Bone & Joint Surg 23 335 1941

Fitte M J Gonzales L A and Bracco A Tratamiento de las escoliosis graves la reseccion de la columna Bol Trab Acad Arg Cirugia p 561 1948

Gonzales I A Escoliosis Rev Ortop Traumat Latino-Ameri ana 1 181 1956

Gucker T Experiences with poliomyelitic scoliosis after fusion and correction J Bone & Joint Surg 38 A 1?81 1956

Haas S L Influence of the fusion of the spine on the growth of the vertebrae Arch Surg 41 60? 1940

Herbert I J Sur le traitement chirurgical des scoliosis Soc Intern Chir Orthop Traumat p 191 1954
Irwin C E Paralytic scoliosis Am Acad Orthop Surg Instr Course Lectures 5 24 1948
James J I P Idiopathic scoliosis Soc Intern Chir Orthop Traumat p 143 1954
——— Paralytic scoliosis J Bone & Joint Surg 38 B 660 1956
Lowman, C H Abdominal Fascial Transplants Ann Arbor Edwards 1954
Marino Zuco C Poliomyelitis Papers and Discussions Presented at the Third International Polio Congress Philadelphia Lippincott 1954
——— Scoliosis Soc Intern Chir Orthop Traumat p 194 1954
Nachlas I W and Borden J N The cure of experimental scoliosis by directed growth control J Bone & Joint Surg 33 A 24 1951
Ponseti J V and Friedman B Prognosis in idiopathic scoliosis J Bone & Joint Surg 32 A 381 1950
Risser J C The application of body casts for the correction of scoliosis Am Acad Orthop Surg 12 255 1955
——— Important practical facts in the treatment of scoliosis Am Acad Orthop Surg Instr Course Lectures 5 248 1948
Risser J C Laudes C H Norquist D M and Craig N A Three types of body casts Am Acad Orthop Surg 10 131 1953
Roaf R Paralytic scoliosis J Bone & Joint Surg 38 B 640 1956
——— Wedge resection for scoliosis J Bone & Joint Surg 37 B 97 1955
Somerville E Rotational lordosis the development of the single curve J Bone & Joint Surg 34 B 421, 1952
Stagnara P and Queneau P Scoliosis evolutives en periode de croissance 27th Congres Franc Orthop Traumat Rev Chir Orthop p 378 1953
Steindler A Feature and course of idiopathic scoliosis Am Acad Orthop Surg 7 158 1950
Stracker O A Skoliosen Soc Intern Chir Orthop Traumat p 165 1954
Wenger L Transversectomy for scoliosis J Bone & Joint Surg 33 A 253 1951

DISCUSSION

Dr Harrington Dr Carlos E Ottolenghi s paper summarizes the present current treatments in the Western Hemisphere and the Continent It is well for the record that this summary go down in history

In the past 10 years I have worked relentlessly on a surgical method (1) to improve the surgical treatment of scoliosis (2) to preserve the integrity of the individual and (3) to support the vital functions of the body mainly cardiorespiratory

I have come to the following conclusions

1 The etiology of paralytic scoliosis is simply paresis partial or complete of the paraspinal musculature All other considerations are secondary

2 Skeletal structure changes are the result of unequal pressures affecting epiphyseal growth

3 Internal spinal support by mechanical means is possible and indicated

In the normal growing child the integrity of the spine is maintained by adequate musculature working through symmetrical skeletal tissue When either of these elements is disturbed potential mal-development is inevitable By the nature of poliomyelitis and its associated paresis the integrity of stability in the spine is inhibited or lost The extremes of deformity that may be acquired by the individual are directly related to the amount of instability and the duration of growth potential remaining in the patient

The ideal treatment must fulfill the following criteria re-establish and maintain stability of the spine The present dilemma is how to fulfill these rigid criteria and not disturb in a major way the growth of the spine

I wish to differ slightly with Dr Ottolenghi that internal fixation of the spine is physiologically and biomechanically possible

The problems of internal metallic spine fixation with dynamic potentialities are

1 Can metal be tolerated by the body?

2 Can metallic compression and/or distraction forces be applied and still remain physiologic?

3 What are the forces that are necessary to correct curvature or prohibit its progression?



Fig 3/5 Showing the strut mechanism that has been in the body for 1 year The top of the photograph reveals the walking hook in place The lower portion of the photograph reveals the distracting force (rod and hook) imbedded in bone

4 Are vital functions affected

5 How may mechanical forces be applied to establish spine stability?

6 Can correcting forces be applied and adjusted with growth?

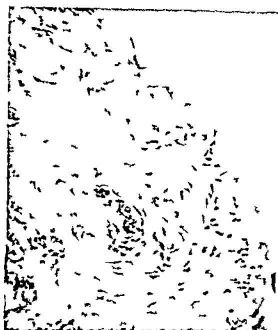
I would like to give you a slide demonstration (Fig 3/5)

A particular reaction of the spine to existence of metal in its proximity—there is apparently acceptance by the body The bone is never in contact with metal There is always fibrous membrane existing between the metallic instrument and the bone

Figure 3/6 shows a membrane removed between a metallic instrument and the bone The bone is not present The membrane in this area is a dense fibrous tissue without polymorphonuclear infiltration This is a photomicrograph taken of a patient approximately a year ago

Figure 377 shows the bone in contact with this membrane and it eliminates the synovial membrane at the bottom Note please that the osseous structure is firm and dense and has changed its character to meet the stress that has been put on it

This is an internal fixation that has been in the body a year and a half There is no foreign body reaction there is no rejection by oxidation there is no rejection by necrosis This is normal tissue enveloping the various elements of the instrumentation



F g 3 6 Photom crograph of the syno al membrane that al ays ex sts l et een the metall c nstrume t and the soft t ssue or bone

In the past 8 years o er 5 reno at ons of pr nc ple and des gn ha e been tr ed to de elop th s p esent concept of the necessary elements to establ sh correct on and stab l ty n a scol o c sp ne

1 There must be a d stract on force and there must be a compress on force These forces must ork n un son and mu t ha e freedom of ac t on to allo to gro v h and mo on yet st ll ma nta n the r ntended funct on of d stract on or compress on

2 On some sp nes n order to acqu re max mum correct on at any one s t ng a d stract on force of o er 13 000 lbs per sq n has been ap pl ed thout dele er ous effect Th s pressure does not rema n constant W th n a few mo ments or at least hours th s force s redu ed to pract cally no h ng by release of the soft t ssue These soft t ssue elements are l as cally respon s ble for the res stance met vhen d s ract ng forces are appl ed

In the past year s work clo ely related card o resp ratory funct on stud es on operat e cases re ealed that all resp ratory tal capac t es ere mpro ed



F c 377 Sho v ng the react on of the transverse process into more dense bone *and the healthy fil rous t ssue that ex sts* l et een the metall c instrument and the l one

Card ac stud es ha e re ealed that scol o cs sho v p olonged mechan cal systole h ch we interpret as a man festat on of card ac wo k under uneconom cal c rcumstances Only me v ll perm t us to substant ate the n al cl n cal mpress on that the correct on of the ana om c deform ty s follo ved by regress on of the ab normal find ngs

In th s ser es of o er 20 scol ot c cases ope a ed on under th s study start ng n July 1956 no case sho ved tal capac ty reduct on No ex ternal fixa on vas ut l zed in any ca e The a erage hosp al stay before release to home care vas 12 days No compl at ons have resulted

T me and ob er at on ll ans er quest ons of the tolerance of th s mechan sm by grow ng bone

True e ha e met d fficult es n the pa t of eros on of bone and fracture of the metall c n struments

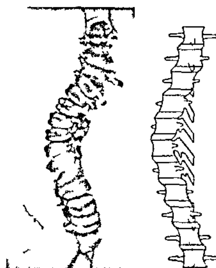
In none of the study opera e ca es n wh h ve used equ pment of the la est des gn has there been eros on of bone allow ng loss of d s tract on force or f acture of the equ pment

In conclus on I bel e e that due to the na u e of paralyt c scol os s the stab l y must be re establ shed n the sp ne dur ng the pe od of gro vth It s poss ble to a compl sh th s by n ernal metall fixa on The me hod that I present s not cons de ed a panacea and s st ll n the final de elopment s ages I w ll show a sl de of a recent case (F g 3 8) and let th s

Fig 3 8 (*Left*) Roentgenogram aken n a and po o ho derately se ere thorac paralyt c s ol o (*Center*) Po oper t e oen nogram he stand ng po on (3 months postoperat e) (*R ght*) l en genog a n e l n d p t on (3 months po toperative)

me hod speak for tself Th s s befo e surge v the surgery durat on was ? hours approx ma ely 35 o 40 m nutes were spent n wa ng allo v ng soft t ssue to release a d stract on force on one s de a compres on force on the other w h final sp nal fus on after a mod fied H bbs and a long graft I firmly bel e e that once the extremely techn cal f ctor of appl ca on of n ternal sp ne fixat on is mastered su g al treat ment of scol os s w ll be the appl cat on of nternal fixat on w thout or w th fus on super mposed fus on be ng d cta ed by the age of the pa ent

Dr Roaf Dr O olengh has r ghtly stressed the two aspects of paralyt c scol os s—instab l ty and defo m y I ha e l ttle or noth ng to add to h s excellent account of the surg cal treatmen of sp nal in tab l ty On e the weak port on of the sp ne has been made stable it is eas er for the adjacent port on of the sp ne to de elop com pensa ory curves the pat ent holds h mself s ra ghter w th less effort and fat gue In add



F 3 9 (*Left and r ght*) Model rep o d c n sc ere sco o s l y r at n deform ty alone hout any element of lateral flex on

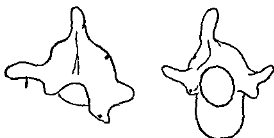


Fig 380 Drawing of a specimen illustrating that the laminae and the pedicles are larger in all dimensions on the convex side at the apex of the curve

tion he has better use of his arms and legs as the proximal limb muscles have a stable instead of an unstable point or origin His trunk and respiratory muscles also act more efficiently All orthopedic surgeons must be agreed on the great benefits of spinal fusion in paralytic scoliosis 5 questions however remain unanswered First how extensive should fusion be? Second at what age should it be done? Third how can development of deformity be prevented? Fourth how can established deformity be corrected? Last after correction and fusion how can relapse be prevented?

I would like to consider the third and fifth questions first as they are closely linked The forces causing deformity will, if uncorrected tend to cause relapse after fusion if the patient is young because the fused spine is young bone and is therefore still plastic I cannot repeat all the evidence now but my own researches have led to the conclusion that imbalance of rotation is the most important primary element in the

Fig 381 (*Left*) Illustrating the action of the longitudinal fibers of the erector spinae muscles which make the vertebrae at the center of their span move forward i e the kyphosis is decreased or in the lumbar region the lordosis is increased (*Right*) Transverse section of normal and scoliotic thorax and the two superimposed Note that in the scoliotic thorax the vertebrae point sideways This means (a) that gravity acts asymmetrically in the vertebra and (b) that the normal action of the longitudinal fibers of the erector spinae muscles in pulling the vertebrae anteriorly now moves them antero-laterally and this movement is no longer counteracted by gravity

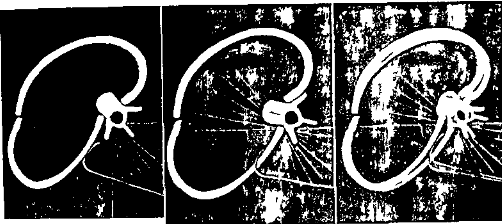


Fig 382 In the scoliotic thorax the head of the rib on the convex side presses posterior to the axis of rotation On the concave side it presses anterior to the axis of rotation as a result the deformity tends to be increased by the rotatory couple of forces Pressure on the concave side or pressure on the convex side decreases the deformity

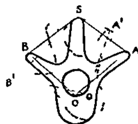
development of severe paralytic scoliosis There are of course mild pure lateral flexion types which are easily corrected and present no problem It is possible to reproduce all the basic features of severe scoliosis by realigning vertebrae in forced rotation deformity without any element of lateral flexion Figure 379 left and right shows this—you can easily do it yourself and make a model and reproduce severe scoliosis without any lateral flexion In addition to the intervertebral rotation there is an intrinsic rotatory deformity of the vertebrae themselves which is largely confined to the neural arch Figure 380 is a drawing showing the hypertrophy of the neural arch on the convex side which is an element in rotation because there is a bigger distance both in the frontal and sagittal planes between the superior and inferior articular facets on the two sides and this would be a bar to reduction

Once a rotation deformity is established 5 deforming forces inevitably come into action which must tend to increase the deformity or cause relapse after fusion

1 The first deforming force is the longitudinal fibers of the erector spinae muscle These fibers normally cause the vertebrae at the center of their span to move forward turning a kyphosis into lordosis (Fig 381 left)

Figure 381 right shows a normal and a

EFFECTS OF ROTATION (II)



In neutral position A B the rotary action of the deep oblique fibres of the erector spinae is balanced ∠SAO - ∠SBO

In the rotated position A B ∠SAO >∠SBO therefore other things being equal the muscles on the convex side act at an advantage

Fig 383 Illustrating the mechanical advantage of the short deep oblique fibers of the erector spinae muscles on the upper part of the convexity and lower part of the concavity of a scoliotic curve

scoliotic spine and the two superimposed Note (1) that the center of gravity lies to the side and does not oppose the longitudinal fibers of the erector spinae muscle (2) that the vertebra

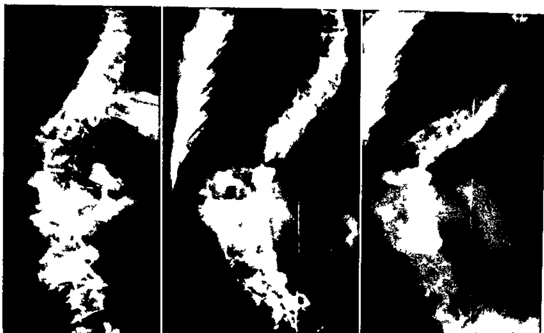


Fig 384 (*Left center and right*) Posterior fusion in the presence of rotation followed by further growth of the anterior vertebral element increases the deformity although the posterior fusion is quite solid

Fig 385 (*Left*) Before fusion (*Right*) Deterioration after fusion

Fig 386 (*Left*) Before fusion (*Right*) Deterioration after fusion

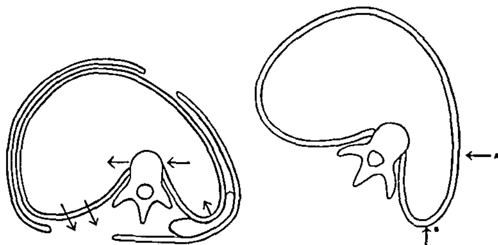


Fig 387 (*Left*) Diagram illustrating the principle in the use of braces to derotate the scoliotic spine (*Right*) Pressure on B tends to derotate the spine but lateral pressure at A may increase the rotation

points sideways therefore when the erector spinae muscle contracts it moves sideways unopposed by gravity

2 The point of pressure of the ribs on the convex side is posterior to the axis of rotation on the concave side it is anterior Therefore the rib pressures create a couple of forces increasing the rotation deformity (Fig 382 left middle and right)

3 The center of gravity lying to one side will tend to produce a secondary lateral flexion and unequal epiphyseal pressures (Fig 381 right)

In addition the short deep rotatory muscles of the spine running from a spinous process to a transverse process lower down act at a mechanical advantage on the convex side in the upper part of the curve Normally the two angles SAO SBO are equal In the rotated spine SAO is greater than SBO therefore those muscles act with a mechanical advantage and incidentally they usually are much larger in bulk on the concave side (Fig 383)

Figure 38? is a picture of the rib cage—you see that the ribs on the convex side are posterior to the axis of rotation If you press on the concave side or if you press laterally on the convex side the rotation is increased (Fig 382 left) If on the other hand you pull on the concave side or press posteriorly on the convex side the

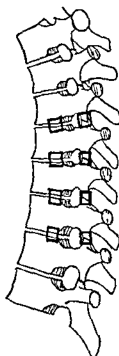
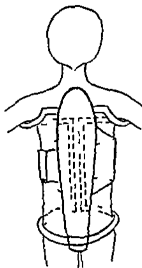


Fig 390 Diagram to illustrate tissue resected at the operation to correct deformity Partial lateral vertebral epiphysiodesis



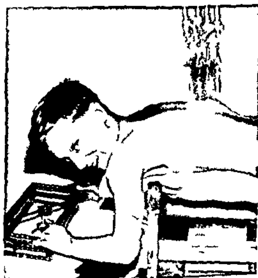


Fig 388 (*Left*) Brace to exert pressure on the rib hump posteriorly and the opposite chest anteriorly

Fig 389 (*Right*) Wire traction on ribs on concave side

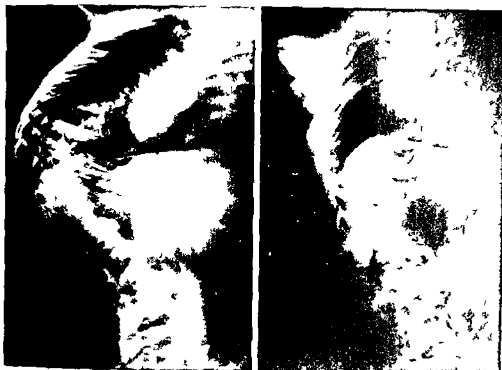


Fig 391 (*Left*) Before operative correction (*Right*) After operative correction

rotation is decreased (Fig 38? middle) Figure 38? right is a superimposed picture of the model in two positions

Now the fourth action is the effect of a laminar fusion on a growing spine The vertical bodies go on growing the graft acts as a tether and therefore if the vertebrae are rotated lordosis is increased which gives the appearance of the x ray increase of lateral flexion and increases the displacement of the vertebral bodies from the midline

Figure 384 illustrates a girl whom we thought we had corrected but we haven t There is too much rotation She was fused The fusion was sound but she grew and 2 years later this is the deformity

Figure 385 is another example This patient also had a laminae neuro-arch fusion in the presence of a deformity She grew and the result was this Spinal fusion in the presence of rotation can have a harmful effect in the growing child

Figure 386 illustrates another case We fused in the presence of rotation the greatest mistake I must confess and here she is ? years later—you see the vertebral bodies have gone on growing and the fused area has not

For those reasons I believe that correction of the rotation deformity—if necessary by operation—should be undertaken in every case of severe progressive or fixed paralytic scoliosis before laminar fusion is performed

Figure 387 shows the principle of conservative correction You ought to press on the ribs posteriorly not laterally—press on the opposite side of the chest anteriorly and pull on the ribs on the concave side to cause a derotation force Now this can be accomplished by braces This is a simple sort of brace the ordinary Thomas brace with pads pressing on the convex ribs posteriorly and the concave ribs anteriorly (Fig 388) You can do it with different types of plasters—there are many ways of doing it You can do it by operation putting wires around the ribs and pulling on them It is quite easy (Fig 389) You see this boy playing about

Fig 392 (*Left*) Before rib resection (*Right*) After rib resection





Fig 393 (*Left*) Before partial resection of scapula
(*Right*) After partial resection of scapula

6 weeks after operation In extreme cases you may have to resect portions of the interlaminate join s and intercorporeal joints where fixed bony deformity is pre ent (Figs 390 and 391 left and right)

As to a_ge of fusion—I am reluctant to per form a posterior fusion below the age of 12 Under that age I rely on plasters or braces and advise the patient to use crutches If the deform ing forces are very strong I perform lateral ver tebral epiphysiodesis (Fig 390) When I do a posterior fusion I fuse the whole of the major curve and 1 or 2 extra vertebrae at each end (Fig 391 left and right)

Finally in the older age group I believe that there is a place for cosmetic procedures or resec tion of r bs and transverse processes Before rib resection you notice the rib hump afterward you can hardly see it We haven t altered the curve We improved her appearance and she was very grateful (Fig 39? left an l right) I have found that this pleases the patients in the older age group

Anoth r form of cosmeti operation is resec tion of the upper part of the scapula the girl in Figure 393 had her scapula stuck up so much that she could not hide it even under the oat she wore After resection of the upper part of the scapula she looked practically normal from the front when suitably clothed

Finally I would like to summarize the key to paralytic scoliosis—the study of the deforming forces—and I would like to say how much I appreciated the honor of being asked here to discuss Dr Ottolenghi s comprehensive and balanced review of an important and difficult subject

Functional Surgery in Patients with Severely Paralyzed Upper Extremities

DR VERNON L NICKEL

When treating patients with severely paralyzed upper extremities the usual concepts of assessing improvement or judging the success of operations in terms of the normal hand should be discarded Otherwise many people with most distressing disabilities will be denied procedures which proportionately would be of great benefit but when considered in comparison with normal function the gain would not be great Such relatively small improvements in the severely paralyzed offer a tremendous step forward in their functional rehabilitation

Extensive time motion studies of prehension show that the major portion of our upper extremity activity is in a rather small area extending from the waist to the face Thus in planning mechanical substitution or surgical reconstruction of these upper extremities we should plan to center function in this area Again if goals are set in terms of normal function—for example elevation of the arm—then because of obvious inability of the surgeon to supply such range the patient will be denied anything

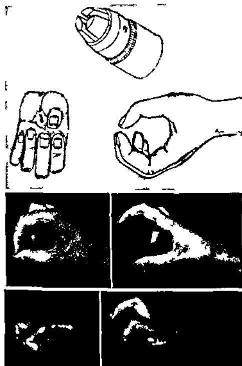
When considering supplying mechanical or surgical benefits to a patient with severe paralysis of the upper extremity we should keep constantly in mind the area and type of activity that is most desirable for this type of patient

A high percentage of patients with severe paralysis of the upper extremities have required or even may still require mechanical respiratory assistance With a low vital capacity this type of patient commonly is denied the benefit of well planned surgical procedures because of the apparent severe risk attendant with any surgery At the Rancho Los Amigos Hospital Respiratory Center for Poliomyelitis surgery on patients with severe respiratory paralysis has been done for over 3 years During the recent 12 month period from March 1955 to March 1956 a total of 264 patients were operated on 25 per cent required respiratory equipment prior to surgery and 52 per cent required respiratory assistance during the postoperative period There has been no operative or postoperative mortality despite the fact that very extensive surgery has been done Only a few relatively minor complications have occurred It is our opinion based on this experience that the severity of respiratory paralysis should be no deterrent in the planning of reconstructive surgery when adequate mechanical respiratory assistance and personnel trained in the use of this equipment are available

Another important consideration in over all upper extremity activity is the correlation of respiratory tolerance with the amount of muscular energy required to perform work We have found patients that are so borderline as far as respiratory capacity is concerned that they severely curtail the use of their arms because of the increased breathing effort required Thus in this type of patient respiratory equipment indirectly will enhance upper extremity usefulness greatly

Increasing interest is being shown in the attempts at mechanical replacement of totally lost function or mechanical reinforcement of less than total paralysis As one works with this type of problem it becomes apparent that whenever possible mechanical devices should be replaced by surgical procedures but that many surgical procedures in the severely paralyzed would be impossible and would not be considered without the knowledge that mechanical apparatus of different types for these irreplaceable functions is available For example if operative reconstruction of a hand is contemplated on an otherwise flail upper extremity elbow and shoulder stability and motion can be provided by mechanical means The next few years will see a definitive improvement in this type of apparatus

Each year surgical procedures that formerly would have been considered quite radical are



F c 394 (*Top*) Illustrat on of flexor h nge hand s mula ng a mechan cal chuck
F 395 (*Bot om*) (*Upper*) A good flexo h nge hand (*Lo e*) Same hand p o to fo mat on of the flexor h nge hand

presen ed th a h gh proport on of good re su s E en more str k ng s the p ogress e n c ea e n the se e y of in ol ement of upper ex em es that a e be ng cons dered for surgery T me p e ents g ng cred t to the many p o nee s whose fundamental contr bu ons ha e made th poss ble

When any transfers are cons dered a full pa nle s range of mot on of the jo nts in ol ed s an absolu e p erequ s te The more se ere the ex ent of paralys s the mo e important it is to adhe e clo ely to th s pr nc ple If spl nt ng cast ng an l phys cal therapy do not ach e e th s su g cal release s requ red befo e further defin e ork s done

The focus of upper extrem ty funct on s the p n h and grasp pro ded by some type of p e hens on Unless some means s prov ded for th s fun tion w ll be mo t l m ted O as onally sub st u on for p nch can be made by de ces that are attached to a spl nt or hand but t must be emphas zed that whene er poss ble we should pro de act e p n h Of course the complete sub t tut on for hand prehens on is the hook used by amputees as a mat er of necess y Few pa ents w th se ere paralys s w ll funct on well ith a hook and the hand tself should be used to supply th s p nch The reason is that the most mportant factor of sensat on becomes more dom nant in the eaker hand In contrast



Fig 396 Illustrat ng the funct on of a flexor h nge spl nt



F g 397 Wr st extens on us ng subl m s to common finger extenso s wh ch p or to transfer ere ent ely w thout fun t on



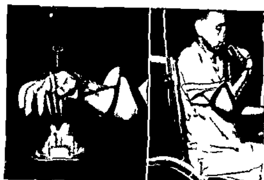


FIG 398 (*Left*) A hand in useful function after supination release

FIG 399 (*Right*) Mechanical splinting to make use of remaining supination power

an amputee usually has adequate strength in the stump and a normal opposite extremity and with this a degree of functional sensation through the prosthesis can be obtained This is not so in a patient with severe paralysis who has to concentrate maximal effort in providing a certain small amount of active motion and by doing this sensation through mechanical apparatus is largely lost

Surgical methods of providing active pinch fall in 3 main categories

1 Tendon transfers of which opponensplasty restoration of finger flexion or extension and so on are most basic and in properly selected cases attendant with a very high percentage of successful results These now well known procedures will not be discussed further

2 Tenodeses active and passive are very useful and should be used when active tendon transfers are not feasible These provide active prehension or in certain instances may be used to provide a passive hook Dr Tyler will present these in his discussion

3 Flexor hinge The employment of this principle is also restricted to those hands in which active tendon transfers are not available and the choice lies between tenodesis and the flexor hinge hand The concept of the flexor hinge hand may be combined with tenodesis

Extensive research of the function of the hand has demonstrated that the major portion of our prehension is done through a mechanism similar to a simple chuck used by mechanics Thus we believe our major effort should be directed to replacing a chuck type of prehension (Fig 394)

A flexor hinge hand is one in which the thumb is stabilized in opposition The interphalangeal joints of the index and long fingers and occasionally of the ring finger are also arthrodesed in a position of function The only remaining motion in this hand is in the metacarpal phalangeal joints which now act like a hinge—therefore the name flexor hinge hand

The flexor hinge hand will give surprisingly good function when muscle strength is minimal A total of 2 functioning muscles which may be quite weak will provide active prehension—1 for flexion and 1 for extension Flexion is provided without further transfer if a profundus sublimis interosseus or lumbricale muscle is present When no active extension is available without or with transfer mechanical extension can be provided and if no muscle power at all is available the concept of the flexor hinge will provide prehension with a minimum of splinting Figure 395 upper and lower illustrates such a hand



Fig 400 Flexorplasty utilizing a transfer of weakened forearm muscles showing range of motion obtained and the principle of active tenodesis to prevent full extension



Fig 401 Flexorplasty with good forearm muscles and poor biceps—an ideal situation for this procedure

In this hand the wrist is stabilized by its remaining power When this power is not sufficient a splint is used The thumb is held in a satisfactory position of opposition by bone block and interphalangeal fusion Interphalangeal arthrodesis maintains position of the fingers and the only motion present in the hand is MP flexion and extension A true type of chuck pinch is obtained (Fig 396)

Prior to any surgical procedures the hand is splinted in this position for a period of time so that the patient knows what function he will have and the surgeon determines by trial and error the optimum position for maximum efficiency

Arthrodesis of the wrist should not be done until prolonged splinting has demonstrated that this procedure will not cause loss of some desired function Of particular importance is the fact that tenodesis procedures depend on wrist *motion and if arthrodesis is done* any such procedures cannot again be considered Active transfers to gain wrist extension are quite effective of which transfer of a sublimis is probably one of the most useful (Fig 397)

If either isolated pronation or supination remains active rigid rotary deformities are very common These may require surgical release if prolonged casting will not correct them Full supination deformities particularly must be brought to a pronated position before the hand can be functional (Fig 398)

When one component of forearm rotation is lacking in the presence of a free range of motion it may be supplied mechanically (Fig 399)

Elbow function will be considered next Again if at all possible surgical procedures should be done to gain active elbow flexion Flexorplasty results in remarkable improvement Not only is active elbow flexion obtained but the transferred muscle across the elbow joint acts as an active tenodesis preventing full extension and thus gaining tremendous mechanical advantage for the weakened muscle to flex this elbow (Fig 400) The triceps muscle is a good transplant to gain elbow flexion (Fig 401)

We would like to emphasize at this point and by this patient, another consideration that is so very important to keep in mind in doing tendon transplantations In most instances two gains are made (1) to remove a deforming force and (2) to gain function In this case the triceps was relatively overactive Any attempt to use the hand was greatly impaired by the triceps forcibly extending the elbow When the



Fig 402 Triceps flexorplasty



Fig 403 The mechanical locking elbow to provide elbow stability

triceps was moved to flex the elbow not only was active elbow flexion gained but hand function was indirectly enhanced as well (Fig 402)

When no active transfers are available to gain elbow flexion the elbow locking device is most useful Flexion force can be enhanced by providing springs or rubber band assists (Fig 403)

Without shoulder stability elbow flexion is of limited value because as the elbow flexes shoulder extension must be blocked so that the hand may remain in the useful range area We have not had any experience with active transfers to stabilize a shoulder This is because in patients who have had severe respiratory paralysis there seldom are available muscles of sufficient strength for transfer about the shoulder girdle However shoulder arthrodesis we believe is of the utmost importance and has not *been used sufficiently Criteria have been established* for shoulder fusion which we think are too rigid Obviously the more complete scapular control results in more motion but even a moderate degree of scapular control will provide excellent stabilization and a reasonable range of functional motion of the humerus Also we believe that it has been customary to fuse the shoulder in too wide abduction in order to gain elevation which as has been pointed out is of limited value Wide abduction interferes with comfortable turning in bed and with crutch walking but most important the hand is not in the optimum position (Fig 404)

Mechanical apparatus can be used to provide shoulder stability as illustrated in Figure 405



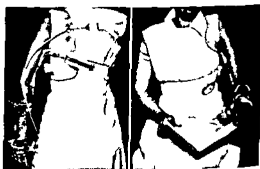


Fig 404 (*Left*) Shoulder fusion with weak scapular muscles illustrating range and stability obtained

Fig 405 (*Right*) Mechanical shoulder stabilization

As we consider the over all picture of upper extremity function we must keep in mind the consideration of trunk stability by casting by abdominal fascial transplants and spinal fusion of scapular control by interscapular fixation and of neck positioning by bracing and fusion—all of which very directly affect the efficient use of the upper extremity in the type of patient we are considering Time prevents elaboration of this point

In summary 4 points have been emphasized

1 In considering the severely paralyzed upper extremity we should assess our results in the light of improvement gained for this particular patient and not in terms of normal function

2 The severity of respiratory paralysis does not preclude major reconstructive surgery

3 The combination of mechanical apparatus and reconstructive surgery is an effective means of supplying basic upper extremity function with emphasis on removing apparatus by tendon or even muscle transfers or arthrodesis whenever possible

4 The flexor hinge hand is a means of providing active prehension with minimal muscle power

DISCUSSION

Dr Campos I am very happy indeed that so worthy and well experienced an investigator as Dr Vernon L Nickel was selected for the presentation of the extremely difficult problem of reconstructive surgery in severely paralyzed upper extremities

His paper was excellent and realistic There is very little to add but many points to focus the spotlight on to emphasize its practical and valuable aspects

The point that impresses one most is the safety with which operations can be performed on patients with severe respiratory involvement which quite often is associated with extensive paralysis of the upper extremities In this particular field nobody in the world can match the experience of the American surgeon who works in the famous Respiratory Centers We just cannot have the same experience because most of our patients with spinobulbar type of polio do not survive the acute stage to undergo later reconstructive surgery due to lack of organization and trained personnel

Certainly one must agree with Dr Nickel that the principles and technics for tendon transfers are rather well established and that the proportion of good results are very high in experienced hands

A reasonably useful upper limb depends very much on a certain amount of shoulder stability Arthrodesis of the shoulder is without doubt an excellent operation and sometimes it is worth while to perform before adolescence since the risks of developing a mild scoliosis are far outweighed by a better functioning shoulder However if after the ravage of the acute stage some muscles are spared to permit an attempt to obtain the active stability of the shoulder joint by the usual methods available they should always be tried because these operations do give quite satisfactory results and besides they do not interfere at all with the possibility of late arthrodesis in case of failure

Among all the operations proposed for paralysis of the deltoid I have had a very gratifying experience with the Leo Mayer transplantation of the trapezius a muscle seldom touched by infantile paralysis

I recommend this operation when I feel it is indicated and I am not too rigid as to the criteria that all the other muscles of the shoulder should be normal In the presence of paralysis or marked weakness of these muscles with subluxation of the scapulohumeral joint a sufficient scapulohumeral stability is obtained with the Nicola tenodesis thus permitting the transplanted trapezius to work and restore to the shoulder some power of abduction

I have found that the resection of the external third of the clavicle and part of acromion eliminates the hindering hump of the shoulder improves the direction of pull of the trapezius and also gives the shoulder a better rounded appearance

Now I am going to show you a couple of cases

Figure 406 left shows a little girl who had complete paralysis of the deltoid and who uses all the other muscles of the shoulder girdle

Figure 406 center and right are photographs made after the trapezius transplantation to the deltoid showing the amount of limitation of the shoulder movements

Figure 407 top left and right are photographs of another patient before and 9 years after the operation transplantation from trapezius to deltoid in a case of complete paralysis of this muscle and with a severe scoliosis The roentgenograms show the range of motion obtained by the operation

There is one last point I would like to make some remarks about The combination of mechanical splinting and reconstructive surgery to improve the function of the upper extremity was very well illustrated and its possibilities at the present time clearly demonstrated by Dr Nickel

Investigations and experiments are being made not only in the field of external mechanical devices but also in the field of endoprosthesis where I believe exists a reasonable basis of hope for the future

Since the early experiments of Lambotte the famous Belgian surgeon to replace the lost function of muscles by internal elastic devices a great deal of work has been done along this



Fig 406 (*Left*) Complete paralysis of the left deltoid remaining muscles of the shoulder girdle normal and normal relationship of scapulohumeral joint (*Center and right*) Transplantation of the trapezius to the deltoid End result 8 months later Excellent stability of the shoulder and satisfactory power of abduction

Fig 407 (*Top left*) Extremely accentuated paralytic scoliosis Total paralysis of the right deltoid The outer third of the clavicle and part of the acromion were resected to improve the line of pull of the transplanted trapezius (*Top right*) End result 9 years after the operation Perfect stability of the shoulder and a reasonable power of abduction (*Bottom*) Roentgenograms showing the range of motion of the scapulohumeral joint after resection of external extremity of the clavicle and part of the acromion

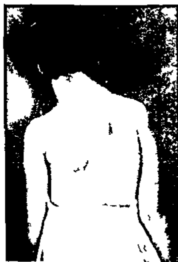








Fig 408 Shoulder fusion

line In a recent pap r from the Norbaka Institutet John Sevastikoglou makes a very interesting review of what has been done in the field of muscle substitution by elastic endoprosthesis and shows the results of his own investigations

We all know and agree that the great hindrance to the replacement of a joint by an endoprosthesis lies in the fact that we have materials rigid enough but not with the elasticity of cartilage to work as a buffer between the frictional surfaces of a joint

On the other hand the replacement of muscles will depend of course on the discovery of coil springs made of elastic materials rigid enough to stand the wear and tear of time and action

BIBLIOGRAPHY

Lambotte A *La prothese elastique perdue dans les paralysies* Bull Acad Roy de Med de Belgique 12 384 1947

——— Note sur l emploi de la prothese elastique perdue dans les paralysies musculaires Bull et mem Soc Chirurgie **180** 140 1934

Sevastikoglou J An experimental attempt to replace muscle function by elastic endoprosthesis Acta orthop scandinav **26** 161 1957

Dr Eyler One need only to have attended previous conferences to realize how difficult it is to present something new and at the same time have it interesting and instructive Dr Nickel is to be commended

In the past many of us have been justly critical of the mechanical and even the mechano surgical approach to rehabilitation of the severely paralyzed upper extremity Most of the function arm braces or ortheses have been bulky unwieldy expensive and more important rejected by patient and parents alike In his thoughtful and instructive presentation at the 1951 Conference Dr Seddon of England voiced the opinion that There is no apparatus for the permanent control of the wrist elbow or shoulder which approaches in usefulness what can be achieved by surgical intervention

Our thinking has been mollified and our practice modified by recent advances in orthotics under the leadership of Drs Shottstaedt Nickel and others Team approach to evaluation prescription fitting and training is proving to skeptics myself included that the use of orthoses on the severely paralyzed upper extremity is an integral part of the rehabilitation of this type patient The motivating mechanism of the Heidelberg arm brings an exciting new facet to the field of orthotics Case selection is a critical factor but anyone who resists employment of such apparatus on grounds of impracticability will sooner or later be guilty of disservice to his patient The goal of course is to eliminate all external assistive and supportive devices whenever possible but the mechanosurgical approach as presented by Dr Nickel is sound

I am always irritated by a discussant who in effect ignores the principal issue and proceeds to present a subject of his own However on this occasion I have been given poetic license by the author

Arthrodesis and tenodesis are the most potent weapons of salvage in surgery on the severely paralyzed upper extremity In most instances arthrodesis is an irrevocable procedure used to stabilize a joint for functional and occasionally cosmetic reasons By arthrodesis one is often able to transfer the allegiance of remaining muscle motors to more distal segments of the extremity Shoulder fusion is a classic example (Fig 408)

Tenodesis is a simple surgical procedure that is both revocable and alterable By definition tenodesis is tendon fixation or suturing of the proximal end of a tendon to bone Tenodesis can be flexor extensor intrinsic or opponens in type Extensor tenodesis of the hand is seldom necessary When the wrist is flexed the hand opens automatically The result of opponens tenodesis often is not worth the surgical effort

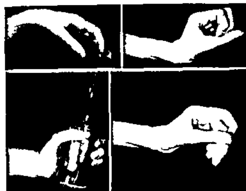


Fig 409 Patient with two tendon hand

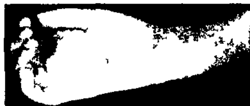


Fig 410 Completely flail hand converted into a useful hook



Fig 411 The flexor tenodesis hook in use

Many methods have been tried but in the severely paralyzed upper extremity bone block opponens gives a uniformly better result Flexor tenodesis provides an excellent method of salvaging function in the flail or partially paralyzed upper extremity The strength of ordinarily non transferable muscle motors can be utilized by tenodesis of the antagonists For example a fair (50 % of normal) wrist extensor ordinarily not considered transferable can be made to serve the dual purpose of wrist stabilization and power for motivation of a tenodesed antagonist In Figure 409 top right and left and bottom right and left we see a patient who has a two tendon hand The forearm and hand are essentially flail except for power in the long wrist extensor and the brachioradialis By extensor flexor intrinsic tenodeses and transfer of the brachioradialis for opposition this hand has improved cosmetically and functionally In the Armed Services it was customary to say that if by reconstructive surgery one was able to give the patient sufficient pinch to hold a quarter and enough grasp to hold a bottle of beverage he was ready for duty

By flexor tenodesis a completely flail hand can be converted into a useful hook with all important sensation Figure 410 shows the hook and Figure 411 the flexor tenodesis hook in use Intrinsic tenodesis will reduce or prevent clawing of the fingers Intrinsic tenodesis can also be used in conjunction with active transfers Limited experience with flexor tenodesis of the elbow has been encouraging and in most cases is better than arthrodesis or posterior bone block at the elbow The principle of tenodesis can be applied by anyone with a good knowledge of surgical anatomy and of course a acute appreciation of the functional problem

Day by day improvement of the respirator has proved to be a two-bladed sword In the past 5 years there has been a marked reduction in the mortality rate of patients severely involved by poliomyelitis At the same time we have been presented with problems for which experts in rehabilitation have little precedent in treatment. Literally to get something from nothing it has been necessary to reassess the surgical mechani

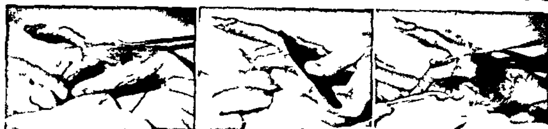


FIG 412 (*Left*) Pull on the extensor digitorum communis tendon causes dorsiflexion in the M P joint but incomplete extension in the interphalangeal joints (*Center*) The same as at the left but at the same time pressure in volarflexion on the I phalanx—thereby the interphalangeal joints extend with good force (*Right*) *Tendon in the flexor digitorum* sublimis inserted in a bore hole in the proximal part of the I phalanx pull on this tendon causes volar flexion in the M P joint—thereby extension in the interphalangeal joints through the extensor digitorum communis

cal and mechanosurgical approach to rehabilitation of these severely involved patients By combining established procedures experience ingenuity trial error and research Dr Nickel has improved upon this concept of treatment Under the leadership of the National Foundation for Infantile Paralysis others are being stimulated to assay and improve on the technics so capably presented by Dr Nickel

DR THIO IASEN The paper presented by Dr Nickel has been a great inspiration to me The fact that Dr Nickel is able to present material of this volume proves that his experience is really great. It is impressive that he has been *able to operate one patient a day of this type* without mortality We have been afraid of operating when patients have had respiratory paralysis and it is always a psychological complication when we want to do extensive surgery on *a child whose life has been saved by respirator* treatment Dr Nickel's results may support us *in our task of persuading the parents to do* surgery on their child

A very important function is the pinch The opponensplasty is a well known procedure but is not always fulfilling the goal I think the function of the abductor pollicis longus and the extensor pollicis longus is very important as basic function for an opponensplasty with the ordinary activation by a crossing tendon from the ulnar edge of the wrist When the function of the thenar muscles is deficient there may be a tendency to flexion in the interphalangeal joint —giving a secondary dorsiflexion in the M P joint The way in which I have tried to overcome this deficiency in extension of the interphalangeal joint is by transplantation of the crossing tendon to the aponeurotic expansion toward the tendon of the long extensor of the thumb The tendon of the flexor sublimis is *split to a point close to the M P joint* of the thumb—one half is conducted below the long

TABLE 181 POLIO EPIDEMIC IN DENMARK 1952 1953

	AGE 0–6	6–15	15 YEARS
Total number of patients with severely paralyzed upper extremities	88	45	100
Treatment for respiratory paralysis with or without tracheotomy	36	23	68
Operations			
Hands (activation)	16	19	21
Elbow (activation of flexion)	6	2	3
Arthrodesis of wrist		1	
Rotation-osteotomy of the forearm		1	

extensor to the ulnar sesamoid bone Here the tendon is looped around the tendon expansion and fixed The other half of the sublimis tendon is looped in the same way around the tendon expansion close to the radial sesamoid bone—the tension must b regulated to give good opposition of the thumb and extension in the distal joint

Paralytic first dorsal interosseus is a problem—the different types of tendon transfer to the lateral band of the dorsal aponeurosis has not been really effective in my hands

The function of the intrinsics is to volarflex the M P joint and to extend the interphalangeal joint If one removes the tendons of the intrinsics and pulls the extensor communis the M P joint will hyperextend and the interphalangeal joints will stand slightly flexed but if one presses the proximal phalanx in a little volarflexion the two interphalangeal joints will extend with good force In the case of paralyzed intrinsics I have taken the sublimis tendon from its insertion and have transplanted the split tendon to the proximal third of the proximal phalanx through a fine bore hole from the radial side The two parts of the tendon are sutured together on the ulnar side It gives a forceful flexion in the M P joint and at the same time by pull of the long extensor a forceful extension in the two interphalangeal joints—thereby giving the index finger the pinch function (See Fig 412 left center and right and Fig 413 left and right)



Fig 413 (*Left*) Patient with severe paralysis in the interosseus muscle and the opponens muscle (right hand) (*Right*) Left hand was primarily as the right The patient has opponens plastic and transplantation of the flexor digitorum sublimis to the I phalanx The patient thereby has a forceful pinch function between the index finger and the thumb

Group and Home Care of Patients With Respiratory or Extensive Paralysis

FRIDAY AFTERNOON, JULY 12 1957

(This Session Convened in the Salle du Conseil General)

Chairman

DR. GINO FRONTALI
University of Rome
Rome

Speakers

DR. KENNETH S. LANDAUER
National Foundation for Infantile Paralysis
New York

DR. JOHN E. AFFELDT
Rancho Los Amigos Hospital
Hondo, California

DR. JOSEPH BENTON
Coldwater Memorial Hospital
New York

MISS RUTH LOCHER
University Hospital
Ann Arbor

Discussants

DR. KARL HARTVIKSEN
Ullevål Hospital
Oslo

DR. KENNETH S. LANDAUER
National Foundation for Infantile Paralysis
New York

DR. J. PEIME
Epidemisjukhuset
Stockholm

DR. GWENDOLYN SHEPHERD
Argentine Polio Foundation
Buenos Aires

A National Program of Respiratory and Rehabilitation Centers

DR KENNETH S LANDAUER

For the most part such patients as we shall be discussing in the next hour formerly would have died Instead they now survive with disabilities that pose a tremendous challenge to us all Having kept them alive we must learn how to provide for them again the opportunities to live as citizens in a free society the opportunities to be useful the opportunities to be happy The program we will discuss was designed to meet this challenge

The National Foundation is a nongovernmental voluntary health agency It is supported by the people of the United States to conduct the battle against poliomyelitis The Salk vaccine is the result of its research program and promises now to reduce the paralytic consequences of the disease but in our country there are still many thousands of patients stricken in former years and unfortunately many thousands of these are very severely disabled It is the obligation of the National Foundation for Infantile Paralysis to help them

In 1949 we conducted a study of the needs of such severely disabled patients In 1950 we initiated a program to establish special respiratory and rehabilitation centers throughout the United States We now have 15 of these in operation established at teaching hospitals of 15 medical schools They range in size from small units of 15 patients to a large center with 160 and total something like 500 treatment and rehabilitation beds for very severely disabled polio patients

In Figure 414 the large triangles show the distribution of the centers of which I speak The smaller dots will give you an idea of the density of the spread of paralytic survivors of poliomyelitis throughout the United States They follow roughly the distribution of our population

In this brief paper we summarize the objectives of this program the concepts guiding its development some of its accomplishments and the implications medical and social and economic derived from our experience of some 7 years

As to needs purposes and objectives—the nationwide study in 1949 revealed a growing number of catastrophically disabled survivors hundreds chronically dependent upon respirators for maintenance of life itself These hopeless patients were scattered over hundred of hospitals where little could be done for them and where they seemed destined to linger through a tragic terminal existence

Factors contributing to this increasing load of seriously disabled patients were the rising incidence of paralytic polio and the rising age incidence in the United States where poliomyelitis is more severe in the older age groups For example two thirds of our patients with respiratory paralysis are young adults Improvements in medical management of acute life threatening forms of poliomyelitis in the United States resulted in a remarkable reduction of case fatality rate which fell from 6 5 in 1949 down to 3 6 in 1956 This explains the rapidly increasing number of tragically paralyzed survivors

It became increasingly important to do something practical positive and a little more intelligent about this increasing load of patients Patients imprisoned in respirators and hospitals represented a human need but there was a scientific and economic need as well

The National Foundation underwriting the care of patients who cannot afford care themselves found in 1950 that it was costing over $3 000 000 to maintain the relative handful of 400 such respirator patients on a custodial kind of care scattered in many hospitals throughout the country A positive approach to this problem was to concentrate these patients in special centers help equip and staff the centers especially for the purpose of conducting care research and teaching and to demonstrate the ever improving patient care that would result This was the genesis then of the center program

Let me discuss the basic concepts and philosophy for a moment The National Foundation invited several medical school teaching hospitals to accept groups of these chronic respiratory

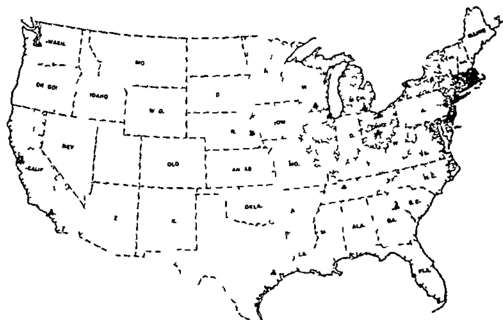


Fig 414 Total number of reported poliomyelitis cases by state 1947 1956 The triangles denote regional respiratory and rehabilitation centers Each dot represents 100 reported cases of polio

patients The plan was to demonstrate the value of the multidisciplinary concept of care to enlist and train allied personnel to encourage dedicated clinical and administrative leadership and to educate professional groups on the value of experimentation in the problems of the chronically hopelessly ill

The first two centers were established in 1950 in Boston and Houston Others have been added gradually as determined by the success of the program and new regional needs Annual supporting grants from the National Foundation have provided funds for key staff personnel special equipment and supplies institutional administrative and overhead expenses In addition the chapters of the National Foundation have paid for the actual cost of the hospital care of these patients and for adaptive equipment Thus we had teams—physicians nurses physical and occupational therapists medical social workers psychologists and others—working with patients grouped in centers who had everything they needed in the way of equipment and other facilities to pursue research and to do the kind of care they wanted to do free of concern either on the family's or the hospital's part about payment of the cost for such care The accomplishments of this program reflect I think the excellence of the medical directors of the center and their associates The medical director at his best has served as the leader of a democratic group which includes of course the patient

The center directors regard multiprofessional staff conferences as indispensable in the planning for total care Doctor nurse physical therapist occupational therapist medical social worker psychologist orthotist engineer vocational counselor and teacher are included Each plays an important role in finding solutions for complex problems Through these conferences the staff can reach agreement on total patient-care objectives and thus maintain a consistent attitude toward the patient and family

Underlying the center's program is the concept of comprehensive care which helps to take into account the patient as a whole human being The physical mental emotional spiritual social and vocational problems are all interrelated The center team is concerned about all

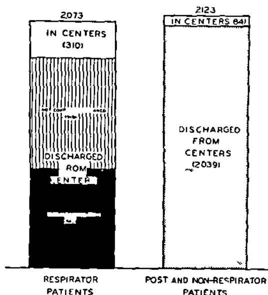


Fig 415 Status of patients ever treated in respiratory rehabilitation centers from 1950 to September 1956

these values and should be able to provide or obtain every kind of help needed No single professional discipline is sufficient The concept of comprehensive patient care implies that all members of the team will search actively for new and better solutions to the problems of the severely incapacitated patients The results of such an attitude are already evident. In 6 years of operations this expanding program of respiratory and rehabilitation centers has freed thousands of such hopeless patients from meaningless incarceration in hospitals It has helped them achieve greater degrees of self sufficiency and independence than had been thought possible

Figure 415 shows that from the inception of the first center in 1950 to September 1956 4 196 patients had been admitted for rehabilitation and 3 802 had been discharged All of them had severe widespread paralysis from poliomyelitis Of the 2 073 admitted in respirators 1 763 had been discharged 845 of them completely weaned from respirator aids The most significant fact the greatest accomplishment of all was that of 918 patients whose lives were permanently dependent upon artificial respiration equipment 92 per cent (or 845) had been released from existence in hospitals to return to fuller lives at home

The average time for rehabilitation of such respiratory patients in the centers was 7 months the average cost approximately $7 000 per patient These patients had been in other hospitals for a year or longer prior to center admission and had become relatively fixed The 7 months period of rehabilitation is indeed a great achievement

In the efforts to rehabilitate respirator patients the majority of whom were essentially quadriplegic the centers also have developed successful new technics and methods that are of benefit to the nonrespiratory severely disabled patients as well

Figure 416 shows that in 1949 at the time we first surveyed the number of respirator patients in the country we had a relative handful 451 of whom only 52 had been able to return home Through the successive years we find a growing number of respirator patients added to greatly in 1952 and 1953 by large epidemics The white column in each instance indicates the number who have been helped to return home even though still needing respiratory equipment The last column shows that by January 1957 there were still 579 chronic respirator patients still in hospitals but I would like to point out that as of June 1957 a great many of these also have been successfully rehabilitated and returned to their homes Thus in the United States today through intensive efforts of these special centers in research and multidisciplinary team care we have found some solutions to problems of the chronic respiratory patient and we have learned how to be of more assistance to the nonrespiratory severely disabled patients

Now let s talk economics for a few minutes If we accept the center directors estimate that about 40 per cent (1 500) of the 3 802 patients discharged would otherwise have continued to require domiciliary care in hospitals or nursing homes for the remainder of life we come to some appalling statistics It is worth speculating on the financial cost of giving merely custodial care to this type of so-called hopeless patient who might as has been demonstrated through comprehensive care be returned home

Assuming conservatively an average life span of only 5 years for these patients and a per diem hospital charge of about $20 it can be seen that the total cost of providing mere custodial care

runs into many millions of dollars We have estimates indicating that dollars spared by this program from expenditures in needless custodial domiciliary hospital care amount to almost 30 million Thirty million dollars are better dedicated to research and teaching than to unproductive custodial type of hospital care

There are costs for caring for many of these patients at home but these are considerably less than in the hospital ranging from one tenth to one quarter of what hospital costs would be Even if the more important human values are disregarded it is evident that the millions of dollars spent on unnecessary custodial care is a serious waste Also it is important to point out that the medical responsibility of the center does not stop when the patient has been discharged home In the next paper Dr Affeldt will indicate the scope of the program in one such center

Another fact of economic significance which we discovered in this program and is important also in every type of chronic illness is that the later in the disease that the patient is admitted for rehabilitation the harder the job the longer the period needed the less the benefits and the greater the cost Tragic and preventable deterioration or dissolution physically physiologically and emotionally starts at the onset in the acute illness in this as well as other diseases Much of this damage becomes relatively fixed in polio in 4 to 6 weeks If comprehensive services can be given from onset of the acute illness only one third as much time and money are consumed and the functional end results are much better

It is important to note that in granting funds to help establish the centers the National Foundation for Infantile Paralysis has provided no stereotyped pattern for their operation They

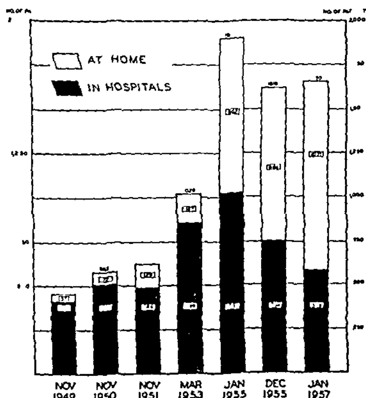


FIG. 416 Number of respirator patients in hospitals and at home based on national surveys 1949-1957

develop their own programs However the National Foundation staff has occasionally been helpful with guidance and advice

It is of interest to note that all of the original medical directors and most of the key personnel on their teams have remained a part of the program and have become more enthusiastic each year This is a remarkable record for programs of care for the chronically ill have been notably difficult to staff with well-qualified persons One suspects that the reason is not merely the support of and freedom to do research and teaching nor the provision of personnel facilities and other resources to do whatever can be done to help these patients Nor is pity the guiding motivation The explanation for this interest would seem to reside in the stimulating effect on the staff of the patients themselves the human values of their achievements their accomplishment of seeming impossibilities despite the enormity of their physical deficits and the tremendous significance to them of even the tiniest functions regained Thus have the patients inspired the center teams in treatment and rehabilitation activities and in their research to find better methods of management

Working in this framework the teams have developed advanced levels of interprofessional understanding and acceptance The various disciplines in their day-to-day group efforts and relationships in patient care research or teaching develop awareness of and respect for what each has to offer and understanding of their mutual interdependence Co-operative relationships develop easily in a permissive environment under good leadership where personal dignity and professional ability are respected Balanced staff patient relationships and integration of services have led to unusual accomplishments by the patients Thus the centers have been able to make an exemplary contribution toward the demonstration of the type of comprehensive care which yields the most successful rehabilitation results

Concept of Patient Care in a Respiratory and Rehabilitation Center

DR JOHN E AFFELDT

There are 15 respiratory and rehabilitation centers for poliomyelitis in the United States Because of the strong support and general guidance by the National Foundation for Infantile Paralysis and because of their common goal their programs are remarkably similar Each has its own personality special interests and talents In presenting the concept of patient care in a respiratory and rehabilitation center I speak specifically of the program at Rancho Los Amigos Hospital in Los Angeles California but these remarks would apply equally well to any of the other centers

This center is located in the chronic disease hospital for Los Angeles County It does not receive acute poliomyelitis patients during the first week of illness but does accept them any time thereafter

The acute cases are treated in the Communicable Disease Unit of Los Angeles County Hospital There we maintain a 3 man clinical investigative team to assist Dr A C Bower and his staff in the care and study of the severe respirator patient. The time between disease onset and transfer to us averages around 2 weeks

The center has been in operation $5\frac{1}{2}$ years Up to January 1957 it had discharged 1 590 patients 56 per cent of whom were in respirators during that admission At present direct care is being given to 770 patients consisting of 140 inpatients 130 home-care patients (defined as a respirator patient at home within Los Angeles County under our care) and 500 active out patients

The program of the center is basically oriented to patient care and rehabilitation with 5 objectives

1 The prevention and treatment of medical complications

2 The decrease or elimination of respiratory equipment

3. The restoration of physical activity and function

4 To return patients to their homes regardless of the severity of their case or the equipment required

5 The establishment of vocational opportunities when possible

The hospital program is divided into in patient outpatient and home-care units The staff for these services is divided into medical dental orthopedic nursing physical therapy occupational therapy orthotic psychological vocational medical social service electromechanical and school services

However the patients are not divided into services the beds are not segregated thus all services are equally responsible for all patients While the patient is undergoing his respirator weaning program and learning glossopharyngeal breathing he also is receiving splints and other orthotic devices mobilization is being achieved by utilizing the standing board walkers and the wheel chair and reconstructive surgery is accomplished or being planned This requires closely integrated work by all services and minimizes their divisions

On admission medical and respiratory evaluation of the patient occurs Routine pulmonary studies consist of measurement of the vital capacity alveolar CO_2 tension (Fig 41?) fluoroscopic study of diaphragmatic motion clinical evaluation of respiratory muscle strength and patterns of use and unassisted breathing tolerance This illustration depicts a nurse on the ward measuring the alveolar CO with the infrared analyzer to give us some of our respiratory data. The respirator weaning program is outlined for the patient This includes the testing and the use of various pieces of respiratory equipment e g cuirass respirators rocking beds and tracheal or mouth positive pressure apparatus Glossopharyngeal breathing instruction is started When abnormal breathing patterns are noted therapy is directed toward re-establishment of a normal pattern the respiratory muscles

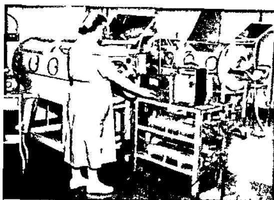


FIG 417 A nurse trained in ventilation studies uses the rapid infrared carbon dioxide analyzer to determine a respirator patient s alveolar CO to be used to guide the respirator pressure and rate settings



FIG 418 A quadriplegic respirator patient with poliomyelitis is placed on a standing board Ventilation is provided by mouth positive pressure using a cuirass respirator pump

are strengthened with resistive exercises mechanical and manual coughing technics are instituted the tracheostoma is evaluated and closed if such appears indicated the pharyngeal and laryngeal *areas are evaluated for function and physical* therapy is prescribed for weakness or dysfunction

The orthopedic staff with the assistance of the physical and occupational therapists evaluate trunk and extremity paralysis deformities and potential function Splints and orthotic devices are prescribed while the patient is still using the respirator The standing board is prescribed the nursing service putting the order into effect Sitting and standing balance is worked on along with specific muscle strengthening Substitute functional technics and devices e g balanced feeder or ball bearing arm support are used as muscle strength permits Figure 418 shows the standing board—a respirator patient on it using mouth positive pressure for ventilation In growing children prevention of scoliosis is attempted by body casts and scoliosis already present is treated by straightening procedures including spinal fusion where indicated The patient in Figure 419 is using a metal halo a scaffolding to allow positioning of the spine *before and after surgery and tracheal positive* pressure for ventilation In such cases the value of combined action by the various professional ervices has demonstrated itself dramatically The joint action of the medical respiratory and orthopedic staff along with the anesthesiologists has made it possible to do extensive cervical and thoracic spinal fusions in completely dependent respirator patients as well as other extensive corrective and functional surgical procedures Another aspect of this professional action is the ability to adapt the patient s physical activity program to his respiratory tolerance and to enhance this when necessary with glossopharyngeal breathing or mechanical respiratory aids (Fig 420)

While the physicians are carrying out or guiding those procedures already mentioned related professional services are also working with the patient and his family In Figure 420 the respirator patient is in the pool receiving treatment again being ventilated by mouth positive pressure The psychologists and vocational counselors are exploring the patient s *background reaction and adjustment* to his situation and his potential vocational opportunities in relation to his present and expected disability Psychiatric consultation is available for the support and guidance of this work The medical social workers explore the family and home situation with the anticipation of mar



Fig 419 A poliomyelitic respirator patient with severe thoracic s oliosis and a flail neck is placed in a metal frame using the metal halo to fix the head and the neck before and after spinal fusion Ventilation is provided by positive pressure to the tracheostomy



Fi 420 A quadriplegic poliomyelitic respirator patient receives physi al therapy in the pool Ventilation is provided by mouth positi e pre sure using a plasti tube which floats on the surface of the water to deliver the pressure from a pump

shalling needed community resources as the time for discharge nears

The weekly patient staff conference is the key to the integration of the program Here all of the services working with the patient meet (Fig 421) to discuss with the patient his present status treatment plan anti ipated goals and time factors

The Home Care Department staff sits in on this conference parti ipating as needed It pi ks up and co-ordinates the compli ated problems whi h go with discharging a patient especially where respiratory equipment is to be sent home The electricians check the patient s home for safety and the adequacy of all wiring doors floors and ramps A separate automatic power generator is installed for safety against power failure Attendant housekeeper service is established with training of the families and employees who will be caring for the patient This department follows and helps the patient at home in routine ways including 24 hour emergency mechani al service (Fig 422)

An important aspect of care is the planned visit away from the hospital for those cases still requiring respiratory equipment The visit begins with a few hours only is gradually lengthened to overnight and then to a week end Su h visitation periods bring to the patient and to his family the realization that e en severely involved persons an be outside the confines of the hospital safely and away from the nurses and doctors This requires careful planning with and training of the family but when the time comes it helps with plans for discharging the patient

Hospital policy encourages periodic readmission for ad litional e aluation and rehabilitation Advan es in rehabilitation technics provide new opportunities for patients once considered static

In addition to the care program research and edu ation are two other phases of the work Clini al resear h is best done where clini al problems arise and where adequate clinical material is available The investigative labora tory staff has been pla ed in the very center of the ho pital area It has rea ly access to clinical problems and is readily available to the clini ians The laboratory group studies the different phases of the respiratory system

The education department provi les training for the profe sional staff as well as for post graduate and un lergra luate students The net result of its ork is to improve our own care program

A subsi liary oncept of the care program both in our respiratory center and others is that



Fig 421 A quadriplegic patient drives his electric wheel chair into the staff conference He uses a mouth stick attached to the controls for piloting the chair The patient has the opportunity to discuss his therapeutic program with the staff



Fig 422 The respirator ambulance delivers a patient to her home as a part of the home-care program The ambulance has its own generator to supply power for the respirator

procedures developed for severe poliomyelitis cases are useful for patients with other diseases The respirator may be lifesaving in other diseases The improved upper extremity orthotic devices for paralyzed poliomyelitic patients are equally useful for arms paralyzed by other diseases

An example of what the center program can do is best illustrated by a 31 year-old male with onset of bulbospinal paralytic poliomyelitis in 1950 He required a tracheotomy and the tank respirator for care His vital capacity had risen to 20 per cent of normal where it still remains He was still using the respirator except for a few hours each day His extremities were non functional and he was completely dependent for all of his care He was discharged to his home with this equipment This all occurred before the center was established at Rancho

He remained at home 2 years with no improvement The home situation deteriorated along with the patient s morale ending up with his return to the hospital in 1953 a bitter depressed man with a divorce pending

The center program had now been in operation a little over 1 year The staff began to work with this patient keeping a close liaison and co-operation between the psychologist medical staff and therapists His first encouraging improvement was learning glossopharyngeal breathing which increased his time out of the respirator and allowed him to sit upright in the wheel chair all day He then learned to use the Robin Aid hook on his right arm and hand using the left shoulder as a source of power With this he learned to write drink from a cup turn pages light a cigarette and dial a phone Walking was tried unsuccessfully but he did gain enough leg function to propel the wheel chair slowly His motivation improved to where he became interested in vocational exploration Progress was slow but by 1956 he had completed a correspondence course on stocks and securities

He has now been discharged to our home care program with an attendant to help him using a cuirass respirator only at night He is employed by a large brokerage firm on a commission basis selling stocks and securities

This patient represents a b fore and after situation as regards the respirator-center program His accomplishments are due to his strong motivation and support from our staff His motivation is due to staff effort as his morale was very low following his first bitter experience at home This case best illustrates what an organized staff and program can do toward the salvage of severely involved and incapacitated patients

Dr Landauer In this report of the work of physicians with their teams to learn more about what might be done for patients catastrophically disabled from any chronic disease it is important to recognize that the opportunities to do research are at the bedside day by day Unless society understands and supports the efforts of these teams in a flexible manner so that they take advantage of the opportunities as they arise we will lose much

Fundamental in our philosophy of grant support to these centers we have built the flexibility to provide for investigation on a permissive opportunistic basis The budgets are such that they permit investigation of various problems when and as they may arise In most countries and certainly our own our schools and hospitals are not adequately supplied with unrestricted research funds to be used for what some call scientific fishing expeditions Our research program in this group of fifteen centers has blossomed I believe through this freedom of operation

Periodically we bring the teams from the centers together to promote co-operation and collaboration These conferences of the groups from the centers on patient care and research problems serve many valuable purposes chief of which is the stimulation of further research and progress toward the ultimate goal of more knowledge better patient care and rehabilitation and teaching The centers have demonstrated the value of this effort so well in their own communities that many of them already are expanding to larger facilities to serve patients with other types of severe chronic disabilities Administrators of public funds are recognizing now the value of investing money in this trinity of research teaching and care They are beginning to recognize that venture capital is necessary when we are dealing with such severe problems and trying to expand the dimensions of good medical care

Dr. Hartviksen It has been interesting to hear the papers of Dr Landauer and Dr Affeldt It is an imposing task the National Foundation has undertaken in America I am especially impressed by what Rancho Los Amigos has managed to do for its patients

We are all convinced of the value of such respiratory and rehabilitation centers as Dr Landauer and Dr Affeldt describe with their trinity of care research and teaching We also surely agree upon the statement that a maximum rehabilitation —a term of Dr Landauer s —of the severely stricken polio patients is an economic advantage to society as a whole and we know too that through our approach to the polio problem we have learned much about the handling of other chronic diseases

Our concept of patient care is almost the same as that we have just heard about and so also is our concept of the treatment We have the disadvantage that in a small unit such as ours we cannot have the highly specialized groups which they have in the centers mentioned Nor can our experience be of their extent However we benefit from having the different specialists available at a modern university hospital such as ours Yet we have the advantage that in such a small unit the staff members get a closer contact with the patients This creates an atmosphere which in itself is the best factor for motivating the patients and also the staff

Our program is in short as follows

1 A respiratory and internal medical program which during the first months will necessarily be the principal one

2 A general muscular re-education program

3 A daily living program during which the different forms of technical aids are tried eventually orthopedic surgery is performed and the vocational possibilities are evaluated In this stage of the treatment the patients return to home is planned Most of these patients need a new house both in order to rationalize the care at home and to give the patients a chance to move about in the flat and to come out

The three parts of the program work together most of the time The stage of the illness and the patients condition will at any period decide which of them will be the principal one

During his stay in the hospital the aim is to give the patient a kind of normal life He shall



Fig 421 A quadriplegic patient drives his electric wheel chair into the staff conference He uses a mouth stick attached to the controls for piloting the chair The patient has the opportunity to discuss his therapeutic program with the staff



Fig 422 The respirator ambulance delivers a patient to her home as a part of the home-care program The ambulance has its own generator to supply power for the respirator

procedures developed for severe poliomyelitis cases are useful for patients with other diseases *The respirator may be lifesaving in other diseases* The improved upper extremity orthotic devices for paralyzed poliomyelitic patients are equally useful for arms paralyzed by other diseases

An example of what the center program can do is best illustrated by a 31 year-old male with onset of *bulbospinal paralytic poliomyelitis in* 1950 He required a tracheotomy and the tank respirator for care His vital capacity had risen *to 70 per cent of normal where it still remains* He was still using the respirator except for a few hours each day His extremities were nonfunctional and he was completely dependent for all of his care He was discharged to his home with this equipment This all occurred before the center was established at Rancho

He remained at home 2 years with no improvement The home situation deteriorated along with the patient s morale ending up with his return to the hospital in 1953 a bitter depressed man with a divorce pending

The center program had now been in operation a little over 1 year The staff began to work with this patient keeping a close liaison and co-operation between the psychologist medical staff and therapists His first encouraging improvement *was learning glossopharyngeal* breathing which increased his time out of the respirator and allowed him to sit upright in the wheel chair all day He then learned to use the Robin Aid hook on his right arm and hand using the left shoulder as a source of power With this he learned to write drink from a cup turn pages light a cigarette and dial a phone *Walking was tried unsuccessfully but* he did gain enough leg function to propel the wheel chair slowly His motivation improved *to where he became interested in vocational* exploration Progress was slow but by 1956 he had completed a correspondence course on stocks and securities

He has now been discharged to our home care program with an attendant to help him using a cuirass respirator only at night He is *employed by a large brokerage firm on a commission basis* selling stocks and securities

This patient represents a before and after situation as regards the respirator-center program His accomplishments are due to his strong motivation and support from our staff His motivation is due to staff effort as his *morale was very low following his first bitter* experience at home This case best illustrates what an organized staff and program can do *toward the salvage of severely involved and* incapacitated patients

DR. LANDAUER In this report of the work of physicians with their teams to learn more about what might be done for patients catastrophically disabled from any chronic disease it is important to recognize that the opportunities to do research are at the bedside day by day Unless society understands and supports the efforts of these teams in a flexible manner so that they take advantage of the opportunities as they arise we will lose much

Fundamental in our philosophy of grant support to these centers we have built the flexibility to provide for investigation on a permissive opportunistic basis The budgets are such that they permit investigation of various problems when and as they may arise In most countries and certainly our own our schools and hospitals are not adequately supplied with unrestricted research funds to be used for what some call scientific fishing expeditions Our research program in this group of fifteen centers has blossomed I believe through this freedom of operation

Periodically we bring the teams from the centers together to promote co-operation and collaboration These conferences of the groups from the centers on patient care and research problems serve many valuable purposes chief of which is the stimulation of further research and *progress toward the ultimate goal of more* knowledge better patient care and rehabilitation and teaching The centers have demonstrated the value of this effort so well in their own communities that many of them already are expanding to larger facilities to serve patients with other types of severe chronic disabilities Administrators of public funds are recognizing now the value of investing money in this trinity of research teaching and care They are beginning to recognize that venture capital is necessary *when we are dealing with such severe problems* and trying to expand the dimensions of good medical care

DR HARTVIKSEN It has been interesting to hear the papers of Dr Landauer and Dr Affeldt. It is an imposing task the National Foundation has undertaken in America I am especially impressed by what Rancho Los Amigos has managed to do for its patients

We are all convinced of the value of such respiratory and rehabilitation centers as Dr Landauer and Dr Affeldt describe with their trinity of care research and teaching We also surely agree upon the statement that a maximum rehabilitation —a term of Dr Landauer s —of the severely stricken polio patients is an economic advantage to society as a whole and we know too that through our approach to the polio problem we have learned much about the handling of other chronic diseases

Our concept of patient care is almost the same as that we have just heard about and so also is our concept of the treatment We have the disadvantage that in a small unit such as ours we cannot have the highly specialized groups which they have in the centers mentioned Nor can our experience be of their extent However we benefit from having the different specialists available at a modern university hospital such as ours Yet we have the advantage that in such a small unit the staff members get a closer contact with the patients This creates an atmosphere which in itself is the best factor for motivating the patients and also the staff

Our program is in short as follows

1 A respiratory and internal medical program which during the first months will necessarily be the principal one

2 A general muscular re-education program

3 A daily living program during which the different forms of technical aids are tried eventually orthopedic surgery is performed and the vocational possibilities are evaluated In this stage of the treatment the patients return to home is planned Most of these patients need a new house both in order to rationalize the care at home and to give the patients a chance to move about in the flat and to come out

The three parts of the program work together most of the time The stage of the illness and the patients condition will at any period decide which of them will be the principal one

During his stay in the hospital the aim is to give the patient a kind of normal life He shall

have a 6-hour treatment in which he is to take an active part This is his work In the evening he receives visits or has coffee with the other patients in the living room or attends concerts shows films or parties These events are now arranged by the patients themselves The social program except for the personal visits if wanted should take place outside the ward

To get the patient and his family acquainted with the new situation the patient goes home for week ends and holidays as soon as possible The stay at home is gradually prolonged This we consider an important part of the treatment

The National Insurance Company pays most of the hospital fees The rest is paid by cities and counties which also pay for braces and splints Technical aids and new housings have until now if necessary been paid by cities counties or funds However recently the government has suggested guaranteeing a certain amount of these expenses

When the vocational problems are too difficult to be handled by our unit we get assistance from the official vocational consultants who are in charge for the disabled in every county We also have special vocational rehabilitation centers However they have not been able to receive the heavily stricken patients but they help us in organizing homework

Our worst problem is to get trained or even untrained help in the house for our patients At our hospital we have a group of nurses who go out by car every morning to give necessary nursing to helpless patients but they cannot stay for more than 1 or 2 hours by each patient So I am sorry to say that in some cases the patient becomes a heavy burden on his family

Dr Landauer has in his paper used the term maximum rehabilitation What exactly does that mean? It is obvious that the interpretation of this term will reflect upon the entire rehabilitation setup and program In the American rehabilitation centers the average duration of the stay of the patients has been 7 months if I have understood Dr Landauer correctly With us it might take months to transfer the patients to cuirass respirators and even longer to wean them from artificial breathing if it is ever possible to do so The different complications might interfere with any effective physical treatment for long periods In the meantime the patients are stiffening up and that which remains or is recovered of motor neuron and muscle activity is undergoing an inactivity degeneration The latent energy and possibilities for active movements can be called upon only through a slow and prolonged pro ess during which the basi strength and tolerance of the patient is built up also Every new muscular function gives a potential chance for yet further functions With our new concept of technical aids where even small movements can manage the more complex control systems activity in practically every muscle might be of benefit to the patient Our experience says that in a quadriplegic polio patient the rehabilitation takes 3 years and even a longer time In fact I would keep the patient in the hospital 3 to 6 months after I have seen the last evidence of progress in him

In order to be able to give all the patients in Norway the same chances of rehabilitation the Department of Public Health has recently asked one of our staff members to take care of the rehabilitation and home care of all the respiratory patients in Norway

Our concept of patient care is nearly the same and that of a national program for the heavily stricken patients is also The differences existing are caused by the differences between a continent like America and our little country as well as by the differences in social legislation

But our aim is as Dr Landauer predicted a maximum rehabilitation of the heavily stricken patients If we can do that we have built a monument which demonstrates the culture of the nation better than any marble pala e

Home-Care Programs for the Patient with Respiratory Difficulties

DR JOSEPH BENTON

In medicine as in other branches of social activity advances that are made frequently create new and significant problems With the development of antibiotics new surgical techniques and advancement in medical and surgical technics the life expectancy of people in the United States as well as in other countries throughout the world has significantly increased This is a wonderful achievement for medical science but has created an entirely new gamut of problems in medicine and in the other co professional disciplines

Recently there has been an increasing awareness of the growing importance of long term chronic illness in an aging population Suggestions have been made throughout the world medical literature that patients with long term illness might best be cared for in their own homes on the basis of psychological as well as practical and financial considerations

I shall discuss briefly some of the factors relating to the origins of home-care programs in general touching upon basic philosophies and then in detail some of the specific problems relating to the home-care management of patients with respiratory insufficiency

Organized home medical services have existed in the United States and other countries for a long time The Boston Dispensary of the New England Medical Center has had such a program since 1796 Similar services were initiated during the 19th century by other voluntary hospitals and independent dispensaries in New York City Among other countries Canada England France the Union of South Africa and Denmark have developed home-care programs as extensions of hospital care

In a recent issue of the *Journal of the American Medical Association* an excellent survey article describing some 31 organized home-care programs was delineated This gives an excellent contemporary summary of such programs from administrative financial medical social co professional vocational and other aspects Those who are interested specifically in this program would do well to read that article

While these general home-care programs for other types of patients and for patients with poliomyelitis have evolved along different types of lines in general the primary concern is with medical care of patients living in their own home Other basic needs such as nursing and social services are provided medications are usually provided also as well as co professional services such as social service physical therapy and occupational therapy In addition housekeeping services and attendant services are also included The program usually operates from a central facility a hospital a local health department a medical college a voluntary health agency or a combination of these Services are usually limited to indigent and medically indigent groups although there are some exceptions —for example programs in Philadelphia and in Montreal where self supporting patients are served by their private physicians on the traditional fee for service basis Well organized home-care programs are now integral parts of the contemporary practice of medicine with special significance for the increasingly important long term chronic diseases

Coming directly to the specific problems relating to patients with respiratory difficulties and home-care programs the basic premises outlined for home care in the other disease entities represent programs of extension of services for long term patients with respiratory difficulties resulting from poliomyelitis as well as other disease entities These have evolved as a natural consequence of the over all comprehensive-care mission of the respirator and rehabilitation centers as outlined by Dr Landauer In general these basic principles have been followed but variations necessitated by the unique clinical problems of patients managed in the centers have been introduced

The long term quadriplegic patient with respiratory insufficiency requiring mechanical respiratory aid poses one of the most difficult rehabilitation problems seen in medical practice As a corollary to this the philosophy and techniques utilized in such patients can be applied to other long term disabling neuromuscular diseases with or without respiratory involvement

Probably the most important feature in home care planning is the appreciation on the part of the patient the patient s family the professional staff and the community that a natural course leading to eventual home discharge is part of the over all scheme for such patients unless specific contraindications exist This premise is of primary importance Many patients find it easy to become psychologically deconditioned to the thought of returning home since the respirator and rehabilitation center programs have been developed to such a degree that many of their medical as well as nonmedical needs have been adequately subserved during hospitalization

Because of the multitude of problems confronting such patients predischarge planning for home care begins early in the course of the patient s hospitalization Home-care plans for the patient with respiratory insufficiency in general differ from home care for patients with other long term illnesses in that more supervision directly or indirectly is required with expedited readmission to a center in case of a medical emergency This point was touched upon by Dr Affeldt This requires a much more integrated relationship to home care on the part of the professional staff of the center *However there are some patients who because* of complete family dissolution due to the catastrophic nature of the disease process will not be able to be returned home These will require indefinite center care not primarily for medical reasons but for social ones

In the predischarge planning for home care center and home-care personnel in joint conferences carefully consider the following specific points

1 *Medical Indications or Contraindications* Where the medical and surgical status is unstable or if the patient requires medical gases such as oxygen home care is contraindicated Aside from these there are no medical reasons why patients cannot be placed in a home-care situation Most patients can return home with respiratory equipment including the full body respirator where this is required In some centers full legal responsibility is assumed for the patient where the program is an extension of center care

2 *Equipment Recommendations* All equipment respiratory as well as other varieties must be on medical prescription Long before home care discharge planning the patient has been studied intensively and his ventilatory requirements are known Consideration for (a) the ventilation and safety factors for the patient (b) the fit and preference of type of equipment particularly for cuirass or shell respirator and (c) mobility factors are carefully evaluated It has been a custom in some centers if a patient does not have at least 2 hours of unassisted tolerance in breathing excluding glossopharyngeal breathing to insist upon home installation of a source of emergency power supply

In addition to the respiratory equipment when indicated in severely disabled patients a variety of assistive and self help devices—hydraulic lifts prostheses splints frames slings wheel chair and attachments mouthsticks reading and electronic devices—are also projected as required Of great importance is the indoctrination of the patient s family and attendant This is actually done in the center while the patient is undergoing treatment Equipment maintenance and operation nursing physical and occupational therapy care and other aspects of patient care are demonstrated to responsible members of the family In addition to instilling confidence this is valuable in that the family is *made to feel a member of the therapeutic team* One center has a regular system of equipment maintenance plan staffed by center personnel

3 *Personnel in the Home* I shall not enter into detail in this except to say that where necessary professional personnel not necessarily a registered nurse is made available to help in the home situation These individuals where families cannot afford the cost are subsidized by payment to the family by the National Foundation for Infantile Paralysis

4 *Vocational Aspects* Patients in centers demonstrate a wide range of readiness for vocational counselling If this is manifested the counsellor sees the patient after he has the established familiar hospital routine and is not acutely ill Testing procedures and interviews

are integrated with nursing and therapy programs and in keeping with the patient's physical capacity The educational process in children or in young adults who might require this is maintained by virtue of established community agencies such as Boards of Education under the special education program which many cities in the United States maintain

5 *Maintenance of Patient at Home* In addition to the regular medical and nursing care the following prescribed physical and occupational therapy programs may be instituted (a) maintenance of range of motion exercises (b) sitting a minimum of 1 hour a day in a wheel chair (c) re-education and strengthening exercises (d) standing for 30 minutes to 1 hour where feasible (e) the continued use of prescribed assistive equipment and (f) activities of daily living (ADL) training including housework simplification for housewives In some centers an attempt is made to readmit the patient for short stays at intervals of 3 to 6 months on an elective basis for re-evaluation of ventilation status and cardiovascular pulmonary renal skeleto-motor skin and other system review Indicated laboratory studies are repeated and the patient is resurveyed to determine his change in status

The mortality rate of this group ranged between 4 per cent and 6 per cent in the 4 year period About half of these deaths might have been prevented by earlier respirator placement when the patient developed respiratory infection

The costs for home care per patient average about $300 to $500 per month depending upon the geographic location in the United States In some areas patients families contribute to these costs in accordance with their ability to pay in others Federal State and local governing bodies assume varying degrees of financial responsibility

Finally the results of such home care programs in one center (a series of 90 patients) were re-evaluated in terms of function 1 year after discharge to home care on a maintenance program Of these 90 at the end of the year of home-care management 88 were sitting in wheel chairs daily 75 were standing regularly and 42 were walking at various levels of proficiency 79 had partial or complete independence in feeding and writing activities and 53 had partial or complete independence in hygiene and dressing

In conclusion outstanding isolated examples of physician lawyer engineer student and housewife patients exist who have been returned to productive existences This aspect holds the greatest challenge for the total-care programs required for these severely disabled patients However it should be emphasized that return to a job is not necessarily the sine qua non of a rehabilitation program since in many patients retraining to an existence of self-care and sufficiency in daily living also must be considered a notable rehabilitation achievement Such individuals can then live in human dignity and be independent for performance of the basic requisites of self feeding dressing and toileting

BIBLIOGRAPHY

Bakst H Domiciliary medical care and the voluntary teaching hospital Am J Pub Health 43 583 1953

Bluestone E M Some advantages of an extra mural hospital program Hosp Management 71 15 1951

—— The principles and practice of home care J A M A 155 1379 1954

Boas E P The Unseen Plague Chronic Disease pp 7 80 New York Augustin 1940

Browne H H Bringing the hospital to the patient's home Canad Hosp 30 35 1953

Council on Professional Practice American Hospital Association Study to Determine Means of Reducing Hospital Costs p 11 12 1957

Desmarais M H Alcock A J and Hilder J A Home care program for respirator patients Canad M A J 75 8 1956

Ginsberg E A Pattern for Hospital Care pp 153 154 New York Columbia 1949

Hospital Council of Greater New York Organized Home Medical Care in New York City A Study of 19 Programs Cambridge Mass Harvard 1956

Hospitalisation a domicile Gaz Med France 60 1031 1953

Jarrett M C Chronic Illness in New York City pp 741 742 New York Columbia 1933

Jensen F Weissbotten H G and Thomas M A Medical Care of the Discharged Hospital Patient New York Commonwealth Fund 1944

Jodin P Home Care Concoms Medical vol 74 pp 1965 1966 Paris 1952

Loeb J F Report of the Department of Medicine Columbia University Bulletin of Information Series 52 No 46 1952

Montefiore Hospital Home Care Origin Organization and Present Status of the Extra Mural

Program of Montefiore Hospital New York 1949

Perrott G St J *et al* Care of the Long Term Patient Source Book on Size and Characteristics of the Problem (Public Health Service Pub No 344) pp 36 37 Washington D C U S Government Printing Office 1954

Phillips H T and Cohn H D The domiciliary care of sick persons as part of a comprehensive health and medical care programme South African M J 28 613 1954

Proceedings of Conference on the Total Care of the Poliomyelitis Patient with Respiratory Paralysis Houston Texas March 9 1956

Reader G G Organization and development of a comprehensive care program Am J Pub Health 44 760 1954

——— Symposium on Extra Mural Facilities in Medical Education Comprehensive medical care J M Educ 28 34 1953

Robbins E E Home Care—Our Experience at the Reddy Memorial Hospital Address to Ontario Hospital Association Oct 28 1953

Rusk H A and Benton J G Rehabilitation Medicine Medical Progress pp 285 298 New York Blakiston 1954

Rusk H A Silson J E Novey J Dasso M M Hospital Patient Survey An Evaluation of the Basic Characteristics Medical Findings and Potential Disposition of the Patients in the New York Municipal Hospital System with Special Reference to the Problems of Chronic Disease and Custodial Care The New York Foundation 1956

Sturges, G Organized Medical Care of the Sick in Their Homes The Hospital Survey for New York vol 2 p 776 New York The United Hospital Fund 1937

Symposium on Home Care and Nursing Practitioner July 1956

Udell L Philadelphia plan for home care of chronically ill persons J A M A 152 990 1957

Waterhouse A M *et al* A Study of Selected Home Care Programs (Public Health Monograph No 35) Washington D C U S Government Printing Office 1955

Wiingaard P Home nursing in Denmark Ugesk laeger 116 570 1954

DR SHEPHERD Dr Benton s fine paper on "Home Care Programs For The Patient With Respiratory Difficulties was sent to me in Buenos Aires for study I found the first part of his article profoundly depressing

Here we were in Argentina swamped with an aftermath of postepidemic patients just pulling out of a 10 year dictatorship that had disorganized the country with no home-care laws for the crippled that could be discovered (though we searched through the library of Congress and inquired at all the different Departments of Public Health that we could think of) and without funds specifically allocated for either home respiratory equipment or attendants and apparently from Dr Benton s article the secret of the amazing results obtained in rehabilitation in the States was the existence of all-embracing programs and unlimited funds These programs went way back to the 1790 s I quote The Boston Dispensary of the New England Medical Center has had such a program since 1796

Funds were apparently completely adequate since State and Federal grants provided money for the totally and permanently disabled The National Foundation paid all the attendants needed

As I said the first reaction to Dr Benton s paper was one of depression but on rereading his article and on studying his analysis of the complicated setup necessary for the rehabilitation of these terribly handicapped individuals the same pattern began to show in print that I had seen 5 months before on visiting the great respiratory centers in the United States and it certainly was not a picture of coldly planned programs nor of millions constantly poured out but rather the image of an amazing group of people with an extraordinary attitude and I say extraordinary advisedly

During last September while visiting the States I was impressed by this attitude of the respiratory team They did not seem to realize that the patients they were treating were quadriplegics with respiratory difficulty

The whole time I was there I didn t once hear the Vale la pena? Para que? No hay nada que hacer! (Is it worthwhile? What for? They haven t a chance) that we had had thrown at us from the beginning of the epidemic even from the lips of those who should have known better The thought didn t even seem to occur to them On the contrary their immediate reaction was Well now what has to be done to get this patient back into meaningful living

I was introduced to completely disabled persons who were earning their own living at home I was referred to colleagues who were back at work in chest respirators and I met others who were gainfully employed though still using glossopharyngeal breathing

On inquiry I found that apparently not all patients were able to return to full employment but the interesting thing was that the group seemed to accept the second best only after a long dedicated trial of every single possibility

Every nation has its outstanding individuals whose elongated shadows as someone has said become living institutions but here were many groups with the same enthusiastic optimistic bracing attitude running through every member of the team

The success of a rehabilitation program certainly demands the existence of a good program but the secret is not in the program but in the obtaining of a team formed by a staff of high quality and high motivation

The persons affected by polio are exceedingly difficult and trying patients and it is all too easy to pass on the responsibility of their care to someone else At these centers I found that the team as a whole shared the responsibility of the future welfare of the patient and that each member was ready to think constructively and originally within the over all plan Sometimes this was even put to one side if it was felt that it was not to the fullest benefit for the patient

At one fine respiratory center for instance I asked why the State Vocational Department was not used The reply was the not polished but certainly graphic It stinks! I don t exactly remember what the difficulty was but it had

something to do with the retraining of a Ph D to cobble shoes Well what do you do? We go out and sell the patient ourselves was the answer

With that warm relationship toward the patient pervading the whole group there are bound to be results

The epidemic that hit Argentina last year with an incidence of over 6 000 paralytic cases (in a population of 18 000 000 inhabitants) brought a terribly high mortality rate during the first 3 months Seventy four per cent of the cases were under 5 years old and 22 per cent in *the Buenos Aires area were respiratory forms*

Though we had been through other polio epidemics (in 1953 1942 and 1935) the number *of respiratory cases never had been so appal*lingly high We found ourselves completely unprepared to tackle the problem The medical group in general was out of touch with the latest developments the hospitals were inadequately staffed and badly run the co-professional services were negligible and respiratory equipment was completely insufficient

We were swamped by the severity of the attack Every other child seemed to have breathing difficulties and since in our hospital alone 30 to 40 cases (paralytic) were coming in per day the situation was desperate We had started *to tracheotomize and hand breathe the children* when the wonderful gift of iron lungs from Uruguay and the States reached us

But not only equipment came

We never can be sufficiently grateful for the help we received from our colleagues from other countries We were overwhelmed by the generosity of those who contributed and by those who came

It was the first time that we had come into the orbit of the help offered by the world medical family and it was a most moving and enriching experience

It was an extraordinary privilege to have the direct advice of consultants from other countries for our own Lauritas Miguelitos and Moniques

The experience so gained is what has helped us I feel to dare to think that in a year we can catch up the lost 10 years when we were isolated from all new rehabilitation technics

However the privilege of having foreign experts helping us led to slight complications and the need for some careful steering when the consultants who visited us in the *morning coun*selled tracheotomy and P.P as the only solution while those who came in the afternoon believed only in rocking beds and cuirass respirators! Many of our patients bear the scars of the two opinions on their necks and on their espinas iliacas antero-superiores! But they are alive and being rehabilitated

Home-care plans for the patient with respiratory insufficiency demand as Dr Benton says more supervision directly or indirectly than with other long term illnesses and an expedited readmission to a center in case of *medical emergency This requires (I quote)* a much more integrated relationship to home care on the part of the center's professional *staff*

Adequate home care of the respiratory patient is based on the existence of a well-organized respiratory center and the creation of such a center is the first step in any home-care program

We had to start from scratch and from data carefully culled from the visiting consultants, a plan for a modern respiratory center was presented to the authorities Since we now have a Ministry of Public Health interested and keen on rehabilitation the program was accepted and our first respiratory center was opened in Buenos Aires last September with a capacity for the treatment of 30 to 40 cases

These last few months have seen the knitting together of the members of the team and the *working out of an adequate balance between* research and rehabilitation (We still have not completely solved the problem created by the *Rehab Group's habit of disappearing* under cover of darkness when the Whittenberger Ferris film is being expounded or re-expounded)

The single most effective measure in focalizing this objective has been the organization of the Occupational Therapy Department under specialized technicians lent by the British Government and by the Rancho Los Amigos Respiratory Center and through the financial aid of the Argentine Polio Foundation (ALPI) We are hoping that the future will see the early setting up of a program for the preparation of specialists in this aspect of rehabilitation

Our psychiatrists have been of amazing help during the epidemic and postepidemic phase and we have learned to depend on them for a

practical solution not only of the patient's problems but also for those that appear through the interpersonnel relationships during a post epidemic

In the past we have had some wonderful cases of total rehabilitation of the severely disabled but these have been isolated incidences and have depended upon the efforts of a disinterested and dedicated physician *In the rest of the cases we have been satisfied with survival*

But survival is not the answer and cannot satisfy us now that we have seen the results of an integrated program of rehabilitation The incredible power of the words we have seen lead us to hope that our patients too will be given the chance of seeing transformed into reality the things that up to now have only been hoped for

Vocational Aspects in the Rehabilitation of the Poliomyelitis Respirator Patient

MISS RUTH LOCHER

The major concern in the early phase of poliomyelitis is survival Even in the early postacute stage the patient is exceedingly anxious about his chances to live Gradually other ideas begin to become conscious fleetingly at first but not long enough to add more worries to his collection of fears and acute anxieties These ideas center on the questions of What shall I be like? What can I do? Can I walk again? If the patient is an adult man one can expect rather soon some expression often indirect of concern about his job or his work We anticipate this thought and try to help the patient talk about it Early in the treatment process the only assurance that can be given is that the matter of work employment or income producing activity will be considered and that every effort will be made to help him become self supporting The staff of the treatment center should recognize that the meaning of work is greater than just a source of income When we are truly aware that being productive has emotional and social as well as economic significance the patient senses the reality of the assurance that has been given him

Along with the many worries the patient has about his condition and the future he also has the immediate problem of the cost of hospitalization and medical care The family too is confronted with this problem and with the loss of current income the situation could be so serious as to interfere with successful medical care We need to acknowledge that much anxiety is eliminated at the beginning when the patient and his family learn that the National Foundation for Infantile Paralysis will give whatever help is needed and as long as it is needed to provide the best medical care and rehabilitation that is available We know that even small children can worry about the cost of medical care and it can interfere greatly with the treatment program One 8 year-old girl suffered severe stomach upsets and headaches weeks after the acute stage No organic basis could be found for it The trouble cleared up when she revealed worries about the cost of care and the fear that her father would need to sell his car—and could not visit her—and his house so that there would be no place to live when she left the hospital When I told her that the National Foundation for Infantile Paralysis would help her Daddy and that there was no need to sell anything she sighed so deeply with relief that the patients in adjoining beds could hear her! With one major worry eliminated the total treatment program has more chance of success

While the patient is experiencing an overwhelming alteration in his body and undergoing strange new procedures in his care others nearby also are having a miserable time No individual lives in a vacuum fortunately and all of us have families with whom we share our lives We need to remember the families as they play a vital part in the rehabilitation of the patient The influence of the family may be the deciding factor in achieving a vocational goal Therefore it is necessary at the beginning of the illness for someone to help the family with the worries that confront them Mothers fathers wives husbands children too—all suffer deeply Once the crisis is past and life is saved the family begins to worry about the ultimate outcome of the disease Future plans are disrupted and uncertain Although no immediate solution is evident at least these concerns need to be recognized and talked about At the same time current problems of living come into view Daily affairs are ignored or shoved into the background with the onset of the disease and continue to be handled on an emergency basis until danger is past Then the reality of responsibilities and obligations hits with full force Fear about the present as well as the future creates additional burdens

The medical social worker meets the family

as soon as possible after the patient is admitted to the hospital This is the beginning of a contact that continues throughout the period of medical care and often after the patient is discharged to his home and community From the start the medical social worker helps the family through the weeks of worry and strain that follow She is prepared to assist them in securing information and help from the doctors and to interpret further to them the various aspects of medical care She also directs them if necessary to the various local resources—social economic religious or educational If the *reactions to the current situation are extreme* the medical social worker in collaboration with the medical staff will attempt to secure psychiatric help for the family

During this period one learns from the family many things about the patient what kind of a person he is what his particular role is in his family with whom he has the closest relationship Later more is learned about the patient particularly in connection with his past activities at school at work at home and in the community We find it important to know how he has faced illness frustrations and achievements in the past All this has real meaning in helping the patient to return to a useful life after the treatment program is completed

Although knowledge of the patient is essential *it is equally important to understand the* family Their ability to face this overwhelming situation to make adjustments in their lives and to utilize to advantage whatever resources are made available to them will give evidenc of the support they will give to the patient when he needs it The staff in the hospital can have a powerful ally in the family group or it is possible that our knowledge of the family will reveal the fact that no help will be forthcoming Under such circumstances total rehabilitation is more difficult and it is better to be cognizant of the situation early

Throughout the convalescent period and the rehabilitation period it is necessary for the medical social worker to establish then maintain a good relationship with the patient As his condition improves some of his attention is directed toward the functional use of his body rather than entirely upon the devastating physical cond tion Some of his fear anger and depression is lessened This change may be slow and while the patient is struggling toward an adjustment the medical social worker needs to share thoughtfully with the rest of the staff the knowledge she attains about the patient s attitudes At the same time all the staff working with the patient must keep ea h other informed of their observations of the patient s rea tions Thus it will be possible to adjust the treatment program to the patient s particular needs He will have an opportunity to try out some of his ideas to test his abilities and to direct his energies toward a practical use The patient will then become an active member of the treatment *plan*

Motivation toward the use of one s capabilities is the sine qua non of rehabilitation Where none exists it is doubtful that even the most expert staff with the be t psychiatric consultation can create it Fortunately most people have a spark and this an be nurtured and stimulated by the rehabilitation group which truly understands the patient his family and the community from which he omes Careful evaluation of the patient s moti ation must be made early When help is needed to promote the patient s drive this help must be constant and a part of the treatment from the very beginning

While the patient is in the hospital major focus is upon him and his progress The family *too receives attention and as a consequen e* gives much help to those providing care for the patient However as the patient moves toward fuller rehabilitation an intimate knowledge of his home community becomes increasingly important Not only will the home-care program depen l upon community support and understanding but vocational plans need to be verified by the community Work that may seem practical and feasibl from a medi al viewpoint—that is to the hospital staff and the patient—may be entirely unrealistic in the particular geographic area in which the patient will live Motivating the patient to achieve something that is neither practical nor possible when he goes home leads only to disaster

For the sake of economy of energy time and money as well as of patient and family morale the medical social worker should become acquainted with the community This means that she must have firsthand knowledge of the home situation the cultural social economic e lu a

tional and employment resources of the community and last but not least the attitudes toward disabled individuals Such information cannot be secured adequately by correspondence or by studying statistics about the area

Let me illustrate briefly why the hospital through the medical social worker has responsibility to understand the community An adolescent from a sparsely populated area was near the end of his treatment program and we planned to send him home Many things needed to be considered Continuing medical care facilities for emergency hospitalization schooling periodic nursing supervision and completion of the vocational plan had to be arranged The family attempted to interpret these needs in their community but met with little success When the medical social worker arranged to spend several days in this small city she learned quickly that the community generally was horrified that the hospital considered sending a young man home when he could not breathe without mechanical assistance and was totally dependent upon others to meet his personal needs First the local doctor had not had experience in caring for such a patient Next the community hospital flatly refused to admit such a patient or even to have a tank respirator in the building The school superintendent was irritated that the hospital would so much as think of risking this young man s life by sending him home The public health nurse refused to take any responsibility for supervising home care However after 2 days of interpretation and discussion the community became most interested and co-operative When the young man s condition was really understood everyone was eager to help him again become a useful citizen and they did so

This same kind of interpretation and preparation is needed no matter where the patient lives It is important to have someone from the hospital who knows the patient and is familiar with his present condition and future outlook discuss these things fully with those who will have close contact with the patient after he returns home Employers too need help in comprehending the patient s situation Their first reaction upon seeing the patient is one of shock and frank disbelief that anyone so severely disabled is capable of working in any capacity Along with the shock there is considerable fear too Explanation of the patient s total situation usually stimulates a willingness to give the patient a chance to prove his abilities

Two other factors are extremely important in reaching a vocational goal First the kind of work the patient does must be of his own choosing A job for the job s sake is not held long Second the job should not be created solely to give the patient something to do "Made work is not satisfying and even though the remuneration is adequate the sense of accomplishment is lacking The resultant feelings of dissatisfaction and discouragement can materially delay or defeat the achievement of real vocational goals and seriously affect the patient s total social adjustment

The patient needs consistent help in thinking positively of future vocational goals Whatever ideas occur to him must be considered seriously and explored Day by day week by week emphasis upon the practical aspects of activity is related to the theme of vocational planning Since our cultural pattern includes economic independence and support of the family motivation toward this end is sponsored by careful and continual discussion of functional abilities and intellectual interests Surprising suggestions can come from patients in these circumstances For example a young man who had been a dairy farmer following 4 years service in the Army finally asked if he could try to operate a sewing machine Had the staff not known or understood this man one look at his frame (6 feet 2 inches high) would have created dismay and aroused question about his personality We could have scoffed at his idea or suggested courses in bookkeeping and accounting or have delayed making changes in the treatment program that would lead directly to his desired activity However as the situation developed efforts of the total staff were directed toward the goal that was requested Wholehearted support and encouragement by a staff that had been kept informed of the man s emotional as well as physical progress led to a new vocation that was creative and financially rewarding Commercial embroidering of athletic and sports wear was a service that proved to be needed by various groups in the community In addition the relationship between this man and his wife was sound and she gave acceptance

and encouragement to the extent that he could feel that he had resumed a masculine role as well as financial support of his family

Planning and developing a realistic and satisfying vocation with the patient requires a thorough understanding of the patient his family and community Selection of employment possibilities cannot be made solely on the basis of intelligence and aptitude tests physical ability or disability Training a man for a job only because he has certain muscles and specific intellectual accomplishments can be a total waste of time Focus on the patient as a person is paramount for success Furthermore the staff members of the rehabilitation unit need to have a common philosophy and the same kind of incentive to help the patient They must speak the same language and understand each other in order to share fully all essential information that relates to the patient's program It is important too that each member of the staff recognize that everyone has something to contribute in this whole program Mutual respect for different skills in the treatment team is as important as having respect for the individual differences in the patients Interdependence of the members of the hospital staff upon each other is as great as that of the patient his family and community When all work together rehabilitation can be complete and the patient can be expected to return to a useful and productive life

DISCUSSION

Dr Pejme The indications for respirator treatment vary at different clinics It follows that the results as to rehabilitation also vary for with broader indications for this treatment the occupational rehabilitation improves

In Stockholm we apply stringent indications for respirator treatment and therefore have rather low figures for occupational rehabilitation In this connection we cite our cases with respiratory insufficiency from the epidemic of 1953 The results with regard to occupational rehabilitation were good in the nonrespirator group but entirely different in the other

The total number of patients with respiratory insufficiency amounted to 144 89 of them were treated in respirators and 55 were not considered to require that treatment (Table 182)

Table 182 Patients with Respiratory Insufficiency by Groups

Nonrespirator group	55
After 3½ years	
Fit	18 = 33%
Partially fit for work	37 = 67%
Total disability or death	0
Respirator group	89
After 3½ years	
Fit	2
Paralysis but fully fit for work	15
Partially fit for work	17
Under rehabilitation	4
In respirator	13
Requires constant attention but not respirator	6
Deaths	32

Patients with respiratory paralysis who undergo vocational training may be classified in three groups

1 Those in whom a satisfactory respiratory function has been restored after grave paralysis

2 Those whose respiratory function is still so impaired that artificial respiration has to be applied for part of each day

3 Those who still have severe respiratory insufficiency

In each group there is the additional problem of the extent of paralysis in other parts of the body Patients with respiratory insufficiency whether of bulbar or spinal origin often have paralysis of the upper extremities too The difficulties of rehabilitation are especially great in these cases and undoubtedly present today our biggest problem in this particular field In those with paralysis of the lower extremities the problem is much simpler for most of them are quite capable of doing useful work from a wheel chair Here the problem is largely one of transport and usually can be solved nowadays with invalid carriages and appropriate technical measures in the home and at the place of work

The paralysis of the trunk itself generally can be overcome In milder cases the patient masters this problem himself in severer cases supporting appliances are required Here a spring reinforced cloth corset generally suffices only occasionally is it necessary to apply a leather corset reinforced with metal bars In rare instances the patient has thoracic paralysis of such type and degree that the spring reinforced corset impedes his breathing If in such cases the rest of the trunk has not been severely paralyzed we have overcome this problem by shortening the corset somewhat if this is out of the question the problem will be more delicate and the possibilities of working in an upright or sitting position will be reduced However in our experience a situation of this kind seldom arises In Sweden we are now trying out a plastic chair fitting with a bowl shaped support for the thorax perhaps it may be of benefit to these patients who are unable to wear a corset

There is no need to consider here the first category of patients—those who later have a fully satisfactory respiratory function—for any other paralysis that may persist will place them in a group where the rehabilitation problems are purely general and not the specific ones we are concerned with here However I shall mention a few cases exemplifying the excellent working performances that have been achieved later on despite severe poliomyelitis in the acute stage (Table 183)

TABLE 183 GAINS MADE BY PATIENTS WITH SEVERE POLIOMYELITIS

Sex	Age	Diagnosis	Tracheotomy Indication	Respirator Treatment	Lowest Vital Capacity	Subsequent Highest Vital Capacity	Occupation etc
♂	24	Polio with paresis bulbar and spinal Bronchopneumonia with atelectasis Myocarditis acute	Palatal paralysis encephalitis	Engstrom 1[illegible] days	1 750 cc	2 900 cc (Sept 1953)	Engineer
♂	3	Polio with paresis bulbar and spinal	Pharyngeal paralysis encephalitis	Engstrom 15 weeks	400 cc	2 400 cc (Feb 1955)	Civil servant
♀	25	Polio with paresis bulbar and spinal	Pharyngeal paralysis	Engstrom 10 weeks	Indeterminable	1 900 cc (July 1954)	Housewife (Childbirth 1956)

The situation is different with the other two groups The most important of these in practice is Group 2 where in spite of everything rehabilitation often can and should be carried through In our experience rehabilitation of patients with diaphragmatic paralysis is well worth while

Diaphragmatic paralysis is virtually never total at this stage yet it may be serious However the *combined effect* of the restored thoracic and diaphragmatic breathing usually suffices for the essential respiratory movements during the day though in some cases it may be otherwise nocturnally Patients of this type are found to make up the bulk of Group 2 for while fully conscious by day they have control of their respiratory *function* but when asleep their breathing is insufficient and the exchange of gases inadequate The expiration is usually affected most with greatly elevated CO_2 values not until later does the O_2 saturation fall according to our experience in Stockholm This state of affairs is especially evident after a prolonged observation period At the Contagious Diseases Hospital in Stockholm we have treated a number of these patients in whom we observed gradually developing fatigue going over into short periods of semistupor Here arterial puncture has disclosed acidosis and elevated CO_2 tension as well as reduced O_2 saturation Respirator treatment with a cuirass or rocking bed has brought about a speedy improvement in this respect even when resorted to only at night

Table 184 shows a woman who had the acute stage of polio in 1953 Three years later we found her fatigued and arterial blood puncture showed pH [illegible] 6 CO_2 *tension* 6[illegible] *mm* Hg After about 4 months in a rocking bed nocturnally the values were normalized or almost normalized At the bottom are the normal values from our laboratory head

One male patient (Table 185) has nevertheless shown persistently elevated CO_2 levels in spite of these measures but after regular nocturnal treatment in a rocking bed his mental alertness at least has been normalized We have transferred him to a convalescent home where he makes small articles from a wheel chair by day and sleeps in a rocking bed at night. He spends

TABLE 184 GAINS MADE BY WOMAN PATIENT

Sex	Born	Polio	Total Plasma CO_2	Blood pH	CO_2 Tension	O_2 Saturation	Vital Capacity
♀	19[illegible]1	1953	Aug /56 64 5 vol % ([illegible]9 mMol/lit)	7 26	[illegible] mm Hg	84%	1956 500 cc
			Dec /56 49 5 vol % ([illegible] 3 mMol lit)	7 41	34 mm Hg	8[illegible]%	
	Normal		50–65 vol % (2[illegible] 30 mMol/lit)	7 35–7 45	35 43 mm Hg	94%	

TABLE 185 GAINS MADE BY MALE PATIENT

SEX	BORN	POLIO	TOTAL PLASMA CO_2	BLOOD pH	CO_2 TENSION	O_2 SATURATION	VITAL CAPACITY
♂	1927	1953	Sept /56 87 3 vol % (40 mMol/lit)	7 20	99 mm Hg	74%	Acute 300 cc
			Jan /57 86 1 vol % (38 7 mMol/lit)	7 21	68 mm Hg	95%	Feb /55 1 200 cc
	Normal		50–65 vol % (22–30 mMol/lit)	7 35–7 45	35–43 mm Hg	94%	June/55 900 cc Sept /56 900 cc

veek ends in his own home without the help of a respirator

As regards Group 3 patients with severe re piratory insufficiency we have in Sweden ittle experience of a restored capacity for work Theoretically patients who are able to use their rms and hands satisfactorily might well do nanual work even if they need respirator aid or breathing but experience shows that such vork is often made impossible in these cases by severe paralysis of the extremities In Stock holm at least we have no patient of this type working largely outside the field of purely occupational therapy However we do have 1 or 2 patients who have been discharged home under permanent intratracheal treatment with a positive pressure respirator and have had to be content with this since they are so paralyzed in other parts that vocational work is out of the question

Final General Session

FRIDAY AFTERNOON, JULY 12, 1957

(This Session Convened in the Salle du Conseil General)

Chairman

DR ROBERT DEBRE
Faculty of Medicine
University of Paris
Paris

Speakers

DR C H ANDREWES
National Institute for Medical Research
London

DR SVEND CLEMMESEN
Kommunehospitalet
Copenhagen

MR BASIL O CONNOR
International Poliomyelitis Congress
New York

Summary on Viruses

DR C H ANDREWES

*It would be impossible in 15 minutes to summarize adequately over 30 papers on viruses to say nothing of the contributions to the discussions It would be especially hard to deal with the excellent papers on tissue culture and on general virology which form such a useful background to the presentations on more specialized themes Therefore I propose to confine my summary to two matters which have been in the forefront of our discussions—the position of the ECHO and Coxsackie viruses in relation to poliomyelitis and properly and inevitably the present status of vaccination

THE ENTERIC VIRUSES

After what we have heard we are beginning to see as a whole a family of viruses which is characterized by a habitat in the intestinal tract of mammals very small size spherical shape resistance to ether and perhaps a definite range of stability to varying pH s Certainly some other viruses such as adenoviruses can be found in feces but it should not be difficult to exclude these from the family of enteric viruses which includes the poliomyelitis Coxsackie and ECHO viruses together with others inhabiting the intestinal tracts of monkeys swine cattle and mice We have learned of and I have already mentioned some properties which they all share They differ as seems to be emerging in varying ability to develop certain potentialities—pathogenicity for the primate nervous system also for suckling mice and again for primate tissue cultures There are thus defined three groups fairly distinct yet with undoubted overlaps in their properties

The Coxsackie viruses as Dalldorf has described have pre-eminently an ability to infect suckling mice the mode of attack being rather different for the A s and the B s The A s—some of them—will grow in and destroy HeLa cells but not monkey kidney cells Still fewer will grow in eggs or affect the central nervous system of primates Coxsackie A 14 however seems particularly apt to do the latter and the Russian polio type IV, which is much the same as Coxsackie A 7 seems to cause a disease like paralytic polio in man Dalldorf suggests that other Coxsackie viruses isolated from stools of polio cases should not be too readily laughed off The Coxsackie B s are a little nearer to ECHO viruses in growing as a rule readily on monkey kidney as well as HeLa cultures Several types of disease in man have been described as being caused by Coxsackie viruses Gear has discussed myocarditis in this connection and Furcolow aseptic meningitis due to Coxsackie B 5

This brings us close to ECHO viruses of which Wenner Melnick and Karzon have described several as causing aseptic meningitis These resemble polio viruses in pathogenicity for primate tissue cultures but not as a rule suckling mice they differ from polio in their much smaller tendency to cause nervous system damage in primates There are some awkward ones particularly the European Type 9 ECHO virus which is antigenically like classical ECHO 9 s but is also related to Coxsackie A 9 and puts down suckling mice Perhaps it will be wise to transfer this to the Coxsackie group yet others remain whose position is still obscure If the O of ECHO stands for Orphan because of absence of association with disease it looks as if fewer and fewer of the ECHO viruses still qualify for continued residence in an orphanage

The properties of typical polio viruses we all know But there are strains which have lost pathogenicity for primate nervous system and even for primate tissue cultures and some which are pathogenic for baby mice though not as a rule causing myositis like the Coxsackie A s I imagine one could present many a virologist with a modified virus and forbidding the use of serologic tests defy him to say whether such a virus should be called polio Coxsackie or ECHO Melnick has suggested that all these viruses might have had a common evolution I would go further and suggest that they are of one rather close family and may have become

separate only lately. Perhaps they may be likened to a mountain range with—from the point of view of what we may perhaps call the nervous primate—three striking peaks, polio viruses 1, 2 and 3. Possibilities of recombinations among them have been suggested. Apart from such a possibility I feel that serious and persistent attempts to suppress some of their potentialities and exalt others might lead to surprising results and convince all that these enteric viruses form a definite family.

EPIDEMIOLOGY

We are getting accustomed to the idea that with improving hygiene we are in danger of having to pay the price for less typhoid and dysentery by suffering more paralytic poliomyelitis in older persons. What is true of polio seems likely to be true also of infections by other enteric viruses. The recent widespread European outbreak of ECHO virus 9—meningitis—warns us of this danger. Virus meningitis, Bornholm disease and other Coxsackie infections may really be like paralytic polio up-and-coming relatively new diseases of civilized communities. By the next Congress we may be talking much more seriously of vaccines against these other virus infections.

POLIOMYELITIS VACCINES

There has indeed been a change in prospects for preventing polio since the Third Congress 3 years ago. The Salk vaccine was then just beginning to be used on a fairly large scale and we knew very little about its efficacy. Now we know from experience during the past 2 seasons that it has proved capable of preventing a lot of paralysis in children. That is something of which the Foundation and its workers can well be proud. The vaccine does not prevent every vaccinated child from getting polio but the vaccine which is 100 per cent perfect exists only in our dreams. Looking back still further to the Second Congress in Copenhagen who could have believed that the tissue-culture work reported there by Dr John Enders would have yielded such amazing results in 6 years not only as regards polio vaccination but in the field of virology generally? I am sure we may expect further exciting discoveries to follow from the new work on tissue culture of which we heard on Wednesday.

There are those who believe that a killed vaccine which can be made perfectly safe can achieve all we need in the prevention of paralytic polio. Others point to the theoretical advantages—and they are many—of an attenuated virus given by mouth. If a course of Salk vaccine will protect children throughout the most vulnerable period of their lives that is surely good enough. Salk has at this meeting adduced forceful arguments based partly on theory and partly on experiment why such a course should be adequate. He maintains that even if antibodies fall off as nearly all antibodies do and even reach a nondemonstrable level yet the tissues remain sensitized able to respond immediately to fresh contact with virus and prevent not intestinal infection but paralytic complications. Others doubt some of these arguments and suggest that there is an element of wishful thinking about them and point out that immunization with other killed viruses does not in fact lead to an adequately persisting immunity. Drs Koprowski and Sabin expect an attenuated vaccine to give more durable immunity with greater freedom from a number of disadvantages. Against this there is increasing evidence from several quarters that the attenuated variants are less stable than was once hoped and that they may revert to greater virulence in the intestinal tracts of inoculees and still more in the intestines of contacts of vaccinees. The proponents of an attenuated vaccine point out that in some less developed countries all children are exposed and develop antibodies to all 3 types early in childhood. The danger of reversion to greater virulence is unimportant there since the environment is saturated with virulent viruses anyway. In just such areas economic circumstances would make the use of Salk vaccine impractical: the results would be so small in relation to cost and there is a crying need for money to combat diseases of far greater importance locally. An attenuated virus would be for many reasons more practicable. It has been suggested by several speakers that there may be a place for inactivated and a place for attenuated vaccines according to the epidemiologic and social background of the country concerned.

About all these things there is really little place for argument. There are two or three important things we don't know and there is

virtual certainty that time—and hard work—will give us the answer We do not know for how long a course of Salk vaccine will give protection against paralytic polio—with or without booster doses That information will certainly come in the course of time We do not know whether or not we can give attenuated viruses by mouth without too great risks from the consequences of reversion to greater neurotropism nor do we know how to ensure that they are not hyperattenuated thus losing their antigenic powers Doubtless in appropriate areas for study that knowledge will be acquired step by step in experiments of wider and wider scope We have two potentially victorious weapons in the fight against poliomyelitis and we shall in due course learn how when and where we can use each of them and whether one is better than the other or they are complementary Six years ago we weren t quite sure where we were going Three years ago we thought we perceived a path Now the road is becoming plainer and more hopeful all the time but it is still not yet a goal achieved it is still a road

Summary on Care and Rehabilitation

DR SVEND CLEMMESEN

It is difficult to give a summary on care and rehabilitation of patients severely stricken with poliomyelitis on the basis of the magnificent papers delivered at this Fourth International Poliomyelitis Conference. I feel humble in this privileged and honored position for which I am certainly not more qualified than any of the members of this Conference. Therefore I shall confine myself to speaking of subjective impressions from this past week and besides try to compare with the Proceedings of the Conferences in New York in 1948, in Copenhagen in 1951 and in Rome in 1954. I still hope that my personal experience will give some color and interest to the viewpoints maintained.

It is a characteristic feature of these poliomyelitis conferences that almost all the papers reveal some personal involvement with the subjects besides the scientific interest. Thus I shall never forget Dr Wilson's introduction here Wednesday in which he openly addressed us to balance our technics of lifesaving with our measures to make the life that is saved worth while and useful. "To give life to the lifetime saved." Here is more than Hippocrates claims! An obligation of honor to the medical profession, a modern ethical medical obligation which will influence our daily work with other patients. In the future it will stand as an unavoidable obligation which we owe to our community and our patients. But how are we able to meet it?

The treatments with hemotherapeutics, antibiotics and artificial respiration of various kinds have during the later epidemics saved so many patients with upper extremity disabilities that these problems have been forced upon us. It is a complicated problem to help these patients along the lines mentioned even if they have no respiratory insufficiency. Dr Landauer has told us of the most admirable American approach to the problems mentioned and Dr Nickel of the constant, not finished evolution of reconstructive surgery combined with mechanical apparatus. Dr Afeldt has told us of the practical work of the rehabilitation team in a respiratory and rehabilitation center and Dr Benton has given us a lot of practical experience on home care of patients with respiratory difficulties. Miss Locher speaking of vocational aspects has given us an instructive lecture in practically applied psychology and Dr Wendland has given us some mental and emotional aspects of poliomyelitis in a little masterpiece of a lecture which should be read by everybody who has to do with the chronically ill children or adults.

No wonder that we have been using the word "care" much more than the word "treatment" during our conferences!

Let us discuss statistics as they have been presented so clearly by Dr Lassen, Dr Hodes and Dr Ritchie Russell. Both the paralytic case fatality rate and the less significant total case fatality rate give us figures now so constant that in the future we shall be able to register the results obtained by vaccination. The later fate of patients chronically dependent upon artificial respiratory aid needs further study so that we can be able to prevent or at least postpone the complications. Further exchange of experiences might be useful here.

Statistics seem to show us the limit of what we are able to do with first-class respiratory aid and we all know that the methods of artificial respiration which have been developed for poliomyelitis are proving to be of great value in saving life in other diseases.

The long list of outstanding papers concerning artificial respiration prevents me from mentioning names of each of the authors. These papers all together form a veritable textbook of physiology and treatment of respiratory insufficiency. I am deeply impressed by this paramount review of applied physiology and technic which has been presented here as well as in the technical and scientific exhibits. The close and well-organized co-operation between the different respiratory centers, the co-operation with physiologists and the free international exchange

of experiences won have given the triumphs On this background the unexpected important finding of the glossopharyngeal breathing technic is a very interesting and encouraging fact beneficial as it is to so many patients

But how should we after all this be able to get further and still better results? The clinicians and physiologists have told us of the different complications which may occur respectively in patients with impairment of control of swallowing of food impairment of respiration and circulatory disturbances hyperventilation and hypoventilation acidosis and alkalosis and so on and how to distinguish between them Still Dr Spencer states that it is entirely probable that the processes of the disease the physiologic effects of treatment procedures and the aggregations of complications have a variable timetable This is the essence of the variability of the disease as the clinician views it Practically the need for prompt syndromic anticipatory treatment is unquestioned As Dr Wilson stated in 1953 and as it is now emphasized by Dr Ibsen the possible respiratory problem should be diagnosed before respiratory insufficiency occurs Increased respiratory effort *should be looked for rather than for the later* occurring respiratory failure

Dr Jungner has carried further the biochemical *investigations and experiences of Bower et al* and Astrup *et al* in his report

The therapeutic range is narrow in the electrolyte *disturbances Both respiratory and meta*bolically conditioned acidosis or alkalosis occur and consequently determination of plasma bicar*bonate alone often will* not be sufficient A possible renal acidosis should be reckoned with The analyzing methods should be decisive in artificial respiration The potassium dosage must be individualized The risk of edema must not be underestimated and yet a high diuresis must be maintained The raised urinary output of calcium and phosphate causing nephrocalcinosis must be counteracted from the start if the later serious complications are to be avoided The genito-urinary and the gastro intestinal complications and their treatment have been described by Dr Sweet and Dr Neu respectively The administration of anabolic steroids in cases of genito urinary complications is new and the treatment of gastro intestinal complications from the start at the acute phase seems to be important

If true anticipatory treatment is to be carried out in these patients we must go to the limits of what we are able to yield within application of physiology laboratory technics and general medicine To all this we must add our utmost within psychological treatment physical medicine and rehabilitation

From conference to conference the solution of the difficult problems concerning scoliosis is progressive Thus Dr Ottolenghi and Dr Bennett have now given us out of their deep experience in practice and literature not forgetting the valuable contributions of the discussant The pioneer period within this subject has come to an end and these two papers together give us a comprehensive understanding of the subject where the individualized treatment of each patient is so decisive for the result obtained Still I have a feeling that the final word concerning *the genesis of scoliosis* and *the causes of scoliosis* has not been said By further study of the development of scoliosis we should be able to influence the prognosis further The difficulty is that often the patients are not transferred to the *scoliosis specialists until they have reached the* late stages of scoliosis when the curves suddenly yield Anticipatory treatment again and an at*tempt to influence the prognosis thereby!*

Through Dr Buchthal s interesting paper concerning prognostic implication of electromyography *we know that the fate of the single* paretic muscle has been determined to a certain degree from the start Also we know that the *counteraction of contractures and disalignment of the lower extremities has been so successful* that we obtain much better results now than we *did only a few years ago and that we must not* be passive and leave the muscles to spontaneous recovery

The importance *of the most meticulous ap*proach to alignment of braces shoes and lower extremities during the growth period must be mentioned *Contractures always mean defeat*

I grew a little envious when I heard Dr Cooksey s paper concerning neuromuscular fac*tors in rehabilitation in which he suddenly in* a classic matter-of fact way renews to us the theoretic background of our practical re-educa

tion of muscles and our counteraction of contractures He builds his reasonings mainly upon the observation that it is the lack of proprioceptive stimuli from proprioceptors and muscle spindles which causes most of the wasting in the muscles It is 50 years ago this year that Sherrington put up the concept of proprioceptors all these small end-organs which secure the whole co-operation within the body

Through anticipatory treatment and close co-operation of all specialists we shall be able to get further in our care and rehabilitation of patients severely stricken with polio In care and rehabilitation and in physical medicine we are accustomed to be in close co-operation with non medical people

As Clemenceau once said War is too important to be left to the generals so the architect of our fight against polio Basil O Connor President of our International Poliomyelitis Congress recently paraphrased him and jokingly said that "science is too important to be left to the scientists This is a friendly challenge but he himself has given us an example of functional idealism and co-operation Through his work in the Red Cross and in the fight on polio he has been influencing our minds and has been the spokesman of private voluntary activity in close co-operation with public health and scientific experts all the time building on the humanitarian instinct of average people Sir you have a unique faith in human resilience and peace! Let me say this before this audience on this special occasion

Closing Remarks

MR BASIL O CONNOR

After those two excellent summaries and what has taken place this week one ought to be able to close this conference with a few words In a sense that would be a little unfair I would say briefly a few of the things that have impressed me as a layman during the four conferences we have had one is the problem that has prevailed for many years the belief that because a disease had a relatively low incidence it had a relatively low significance That kind of thinking has been due to the fact that distinction was not made between the incidence of a disease and the value of the study of that disease Conceivably less value might come from the study of a disease of high incidence than might come from a disease of relatively low incidence These four International Conferences have demonstrated clearly that in the study of a relatively low incidence disease namely poliomyelitis a value can come in no sense comparable with the incidence of the disease itself

Those of us who have followed the developments in what we call in my country after effects care and research in after-effects realize that the whole activity has been one relating not only to disablement by a particular disease such as poliomyelitis but also one that has redounded to the benefit of all disabling diseases and such situations as respiratory conditions not necessarily connected with permanent disability

Those of us who have followed the scientific research in the fields of the viruses come readily to the conclusion that we have practically covered the field down to almost the basic issues of life the composition of the cells the changes of cells and into the fields of genetics All of that study while carried on to find a solution to the problem of poliomyelitis has a value far beyond a value that results in helpful solutions of other disease problems diseases other than poliomyelitis I hope that never again will scientists or laymen feel that the amount of money or the amount of time or the effort spent in studying a disease of low incidence has a value only comparable with the size of the incidence of that disease

This conference has been increasingly more interesting than the third and the second and the first as it should be An unusual amount of effort goes into these conferences in their scientific preparations I speak now not of the practicalities connected with the arrangements of such a conference but in the preparation of the contents of the conference After all a conference is to be judged by its content and its value if any to the public The content of this conference was prepared carefully by a program committee of distinguished men to whom I wish to pay my respects and to offer your thanks as well as my own The conference has been conducted intelligently and well If there be some who feel that in all meetings international or national discussion seems to be increasingly discouraged we can say that we had a little more discussion at each of our conferences than we had at the preceding one and that we may hope that at future conferences we will have still more discussions

We leave here feeling that real progress has been made in the possibility of preventing paralytic polio with an already existing killed virus vaccine We are well aware of things that we would still like to have solved and explored further

One of the questions about which we are not at all satisfied as yet is some method of determining and establishing the adequate potency of the vaccine so that what we know takes place now in some places can be eliminated Only a layman would dare to make the statement that there is no reason why this kind of a vaccine should not be approximately 100 per cent effective if it is made properly which includes having the proper potency We should not be satisfied with something like 75 or 80 per cent

Another problem that we leave still in an unsatisfactory condition is the question of the application of this vaccine—to what extent it

should be applied throughout the world to whom it should be given how it should be given where it should be given and the proper spacing of it We still have the question as to the possibility of further types of poliomyelitis and as already has been said whether or not it is of another type If it does the same kind of damage we should feel the same kind of responsibility toward it Finally but not intending to include all of the things still left for consideration we would like to eliminate the monkey and get out of what we call in my country the monkey business

Since I know that you feel as we of the United States feel that the elimination of paralytic polio in one country only is not the goal that is sought I repeat that there is a likelihood that we will have eliminated paralytic polio in the United States by 1958 That is not the goal of any science and should not be that is not the goal of the International Congress The goal should be the elimination of paralytic polio throughout the world If that is to take place over whatever reasonable period of time is necessary to accomplish it we might well consider the possibility of joint consideration of such problems from time to time by a small international group indicated by the International Congress In that way not only the solution of these problems might be expedited and their solution more readily agreed upon but also the value of an effective vaccine that can be given to the people of the world to the extent they wish to take it.

As I close this conference I again thank all of you for being here It is the people who come to these conferences that make the conference

Also I want to thank our Swiss friends and the officers of this conference and I want to thank particularly Dr Crasset for all the fine work the committee has done that made it possible for you and me to spend our time here profitably

I wish you a happy and safe journey home

Closing Comments of Chairman

DR ROBERT DEBRE

After the words just pronounced by Mr Basil O Connor it now falls upon the chairman of your final meeting to say a few words I have little to say after hearing Mr O Connor s statement

The importance of the work reported and discussed here has already been mentioned I should like to add that a victory has been achieved by establishing the value of the inactivated virus which was introduced by injection

Here I should like to say a few words to our President Mr O Connor and the National Foundation I should like to express to them our sincere appreciation for the great assistance the Foundation has given us which has permitted us to obtain these results during these great campaigns which were carried out in the United States of America The participation of the population and of the physicians was obtained in an experiment which is unique in its times and has made it possible to affirm the value of the vaccine prepared by Jonas Salk The immunity it confers has been demonstrated in the course of this meeting Those who at the beginning were disturbed by incidents with which you undoubtedly are familiar may today recall what happened at the time of the Lubeck incident, when the error of a physician disturbed minds regarding another vaccine Today we all know the value of the inactivated Salk vaccine and we know about the immunity it confers We know that it develops antibodies which can protect the nervous system but we also know that we still have much to learn We need to know the percentage of subjects that have been protected we need to know the degree of protection that is afforded by this vaccine and we need to know about the duration of the protection

Lastly the techni of manufacture is never completed and you know here again that a certain number of authors such as Dr Lepine have insisted on a certain number of questions Acknowledging the success which has been obtained by the injection of inactivated virus I should like to pay tribute here also to those authors who have worked on the problem of the attenuated virus It is important to remind you here once again of the interest of the infection which is not apparent which is nonpathogenic and which imitates the natural infection The interest resides in the studies performed by men like Koprowski and Sabin which show the great variations of the pathogenic power of the virus resulting from mutations in the human body the interference which may result and the competition among the viruses in the human body and lastly a great and major interest may reside in tissue immunity which is different from that mediated by antibodies

As the problem of vaccination has advanced and progressed the problems of public health which result from poliomyelitis as Mr O Connor insisted are far from being completely solved Its epidemiology is still obscure How does the germ spread? How does it go from one man to another man? How with an infection which is so widespread which is so benign is it possible that some unfortunate human beings become victims of neurologic complications? Lastly as far as public health is concerned another problem arises again and that is the problem of inaccurate diagnosis It concerns the distinction between diseases which are due to a polio virus and those caused by other viruses to which Dr Andrewes referred a few moments ago The Coxsackie viruses the ECHO viruses all of these are widespread and some are involved in certain morbid disorders such as the Bornholm disease But it is impossible as yet to define the exact role that these particular types of viruses play nor can we determine anything so far about effects they may have in certain cases of paralysis This shows you the tremendous field that is still open to the workers over the world today

But as Mr O Connor has said it is possible that from polio we have turned today to a study

of a biologic and fundamental behavior of viruses the phenomena of biosynthesis how it is that an infecting particle actually establishes itself with its acid and its protein and how this little particle infects a new cell In the course of this conference we have heard much about the common properties of various viruses far removed from one another such as tobacco mosaic virus the potato virus and all the various enteric viruses including polio virus itself Here chemistry and the chemistry of biology have certainly become the subject of this conference in addition to polio Thus we have expanded in a very unexpected manner the field of our knowledge and research

I will not speak about rehabilitation nor say anything about the assistance to be given to respiration Of course the truth is that a good pupil can follow only one class at a time and therefore he cannot be considered guilty for not being at two places at the same time

Fortunately Dr Clemmesen has summed up for those who followed the study on viruses precisely what had been said in connection with respiration and also in connection with motor trouble and disorders

I have spoken too much at length and I ask your forgiveness As the Chairman of your last session it is my pleasure and privilege at this time to pay tribute once again and never enough to our hosts in Geneva to Professor Grasset and his assistants to the professors of the Faculty of Medicine in Geneva the authorities of this canton of the city of this small and noble republic It is up to me also to thank on your behalf the National Foundation for the great effort that it has made for the preparation the organization in fact the success of this conference and at the same time to ask that Foundation some day to have the pleasure of meeting with them again The last session of the Fourth Poliomyelitis Conference is hereby closed

Index

